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TECHNICAL REPORT 65

BIOLOGICAL SAFETY EVALUATION OF A COMMERCIAL VACCINE PRODUCTION LABORATORY

Manuel S. Barbeito

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UNITED STATES ARMY
BIOLOGICAL LABORATORIES
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Fort Detrick, Frederick, Maryland

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BIOLOGICAL SAFETY EVALUATION OF A COMMERCIAL VACCINE PRODUCTION LABORATORY

Manuel S. Barbeito

Industrial Health and Safety Division
DIRECTORATE OF INDUSTRIAL HEALTH AND SAFETY

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FOREWORD

A contract was awarded to National Drug Company at Swiftwater, Pa., a division of Richardson-Merrell, Inc., by the U.S. Army Medical Unit to develop and produce vaccines for use in humans. The type of vaccines produced is to be governed by the U.S. Army Surgeon General. Among many other problems associated with vaccine production for human use is the ever-present problem of occupational illness among laboratory personnel. In order to control the potential hazards to workers, the building was designed, constructed in 1962-1963, and equipped in accordance with presently acceptable design features for infectious disease laboratories. However, equipment designed and built with what appears to be desirable features is not necessarily biologically safe. The assistance of Industrial Health and Safety Division, Fort Detrick, Maryland, was obtained to locate and evaluate the hazards of working with pathogenic microorganisms.

Assistance and guidance given by Dr. A.P. Wedum and Mr. G.G. Gremillion in the planning and evaluation of the microbiological tests, and their editing of the text, are sincerely appreciated. The author is indebted to Messrs. A.B. Dove and J.E. Main of Fort Detrick for their technical assistance and to Messrs. J.W. Miller and J.H. Detrick of National Drug Co. for their cooperation in performing the actual tests, without which the data could not have been obtained. Guidance from others when necessary was obtained and although they are not mentioned by name, their assistance is gratefully acknowledged.

ABSTRACT

Operation of buildings and equipment for work with pathogenic microorganisms often involves inherent hazards unknown to personnel responsible for design, construction, budgeting, or research. To a significant extent the facilities and equipment of a laboratory building will aid or deter efforts of operating personnel in maintaining good environmental control and in preventing laboratory infections. The tests reported here were designed to evaluate the microbiological hazards associated with equipment, general building design, construction, operational features, effluent treatment system, and routine research operations in a newly constructed vaccine production facility. The testing procedures and equipment used are described or referenced and the results are tabulated to show the method used to assess the microbiological hazards.

The risks to personnel are characterized, and methods are recommended to improve operation of the building as designed, to modify equipment, and to eliminate hazards.

DIGEST

Simulant microorganisms were used to determine the biological operating efficiency of an infectious disease building that is to be used in producing human vaccines. Biological safety tests and evaluations reported here were designed to: (i) protect laboratory workers against occupational illness, (ii) protect the surrounding community from escape of pathogenic microorganisms, and (iii) prevent the accidental discharge of pathogenic microorganisms into normal effluent waste systems. Challenge concentrations used to determine biological efficiency of equipment or procedures generally exceeded the estimated normal operating amounts in order to demonstrate hazardous conditions, and to insure recovery of microorganisms so that comparisons could be made.

The scope of this report includes: (i) testing procedures, (ii) design features of equipment, (iii) building design, (iv) sampling equipment used for recovery of microorganisms, (v) disseminating devices, (vi) hazards associated with microbiological safety cabinets, (vii) efficiency of cabinet and exhaust filters, (viii) operational aspects of exhaust systems, (ix) evaluation of the contaminated effluent waste system, (x) hazards associated with a refuse incinerator, (xi) hazardous laboratory techniques, (xii) effect of electrical failures, (xiii) building sterilization, and (xiv) other general features associated with evaluating an infectious disease laboratory.

The microbiological safety testing showed that the cabinet and plenum exhaust filters are removing aerosolized microorganisms from the exhaust air at their designed efficiency. The ventilation system is adequate when functioning in accordance with its designed air flow, but any imbalance could create a hazard to operating personnel. Other potential hazards that could result in an occupational illness were demonstrated, such as those resulting from the rupturing of an exhaust duct, the release of microorganisms from an exhaust booster blower for unfiltered animal cages, a common laboratory accident, the pouring of infectious materials down an open drain, and working in a microbiological cabinet under various closure conditions.

Effluent containing vegetative microorganisms was sterilized at 102 C in less than fifteen minutes. However, sterilization of effluent containing spores was not conclusively determined because conflicting results were obtained on repeated tests.

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I. INTRODUCTION

The vaccine production facility at National Drug Co., Swiftwater, Pennsylvania, is a brick and masonry three-level structure (Fig. 1). The laboratories are located on the first floor; the second level ("crawl space"), which is concrete slabbed, illuminated, and vented, houses all ducts and pipe systems (Fig. 2); the third level ("attic") houses filter plenums, blowers, stills, and other necessary equipment for operating the building.

The offices, lunch room, conference room, and boiler room, which houses an emergency diesel-powered generator, are located on the first floor adjacent to the laboratories. A small brick structure housing two 5,000-gallon glass-lined tanks for sterilization of all infectious effluent waste is located near the laboratory.

The laboratory area is divided into five suites, four of which are to be used for vaccine production. The fifth is for rearing and holding clean* animals.

Clean corridors surround each suite on three sides (Fig. 3). Viewing windows to each laboratory room face the corridors (Fig. 4) and are used to observe cultures, animals, and equipment and for routine fire inspections. Two through-the-wall autoclaves and a UV pass box (Fig. 5) are centered in each suite and connect with the interior corridor that serves as a support area for all suites (Fig. 6). Intercommunication is available between the service area and suite and between the suite and air-lock areas located on the end of each suite. A communication page system is interconnected with the telephone and a centrally located public address system.

The exterior walls of each suite contain service panels (Fig. 7) to permit repairs and control of utilities without having to enter the suites. Additional light switches are located near each viewing window so that illumination of the laboratory may be controlled externally for routine security checks.

Air for ventilation of each laboratory room enters at the center of the ceiling through a diffuser surrounded on two sides with windows that cover fluorescent lights housed in the crawl space (Fig. 8). These windows and ventilation duct are sealed and serviceable from the crawl space (Fig. 9). The supply ventilation, both temperature and relative humidity, can be controlled and checked externally from the attic (Fig. 10).

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* Clean as used in this text applies to noninfectious areas or normal animals.

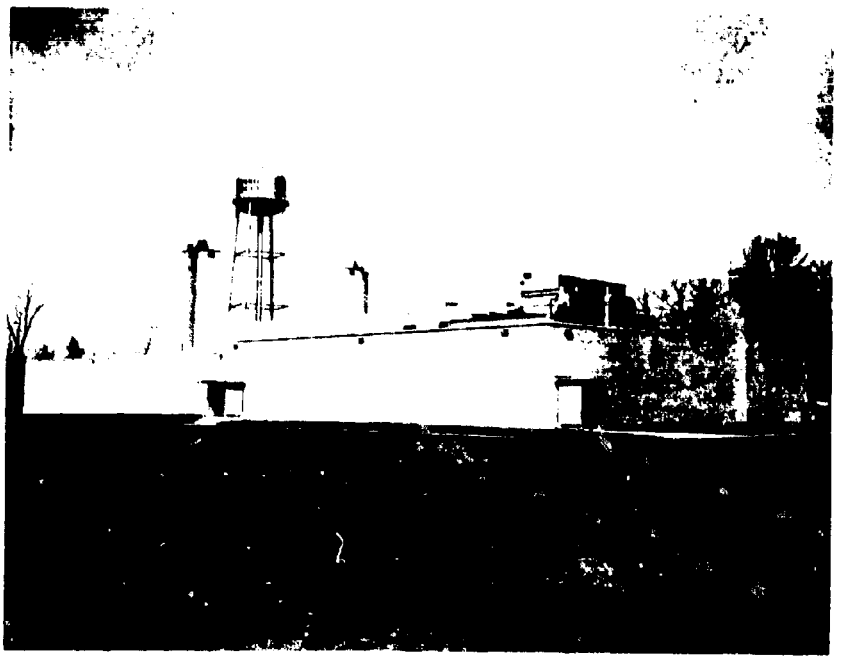

Figure 1. The National Drug Co.

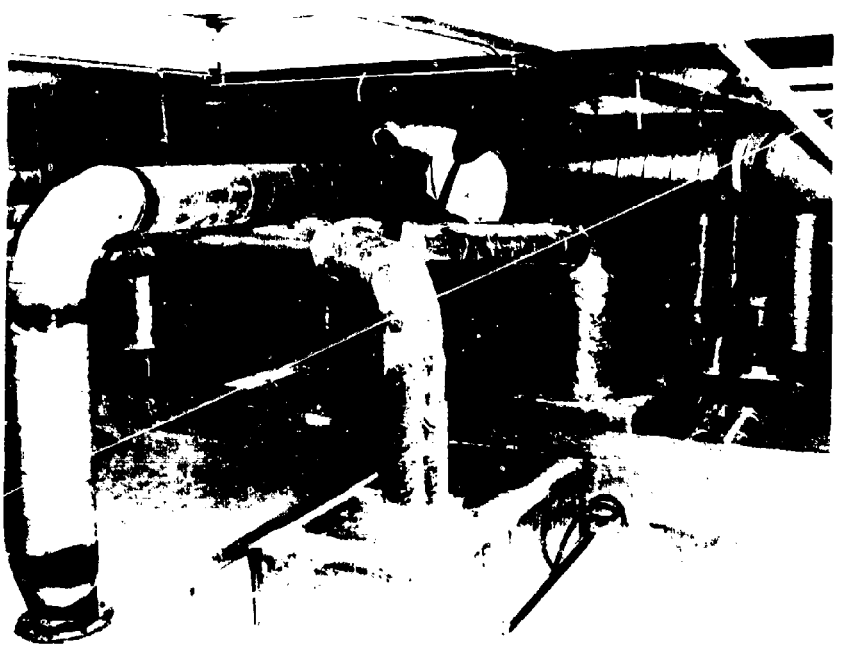

Figure 2. Second Level - Crawl Space.

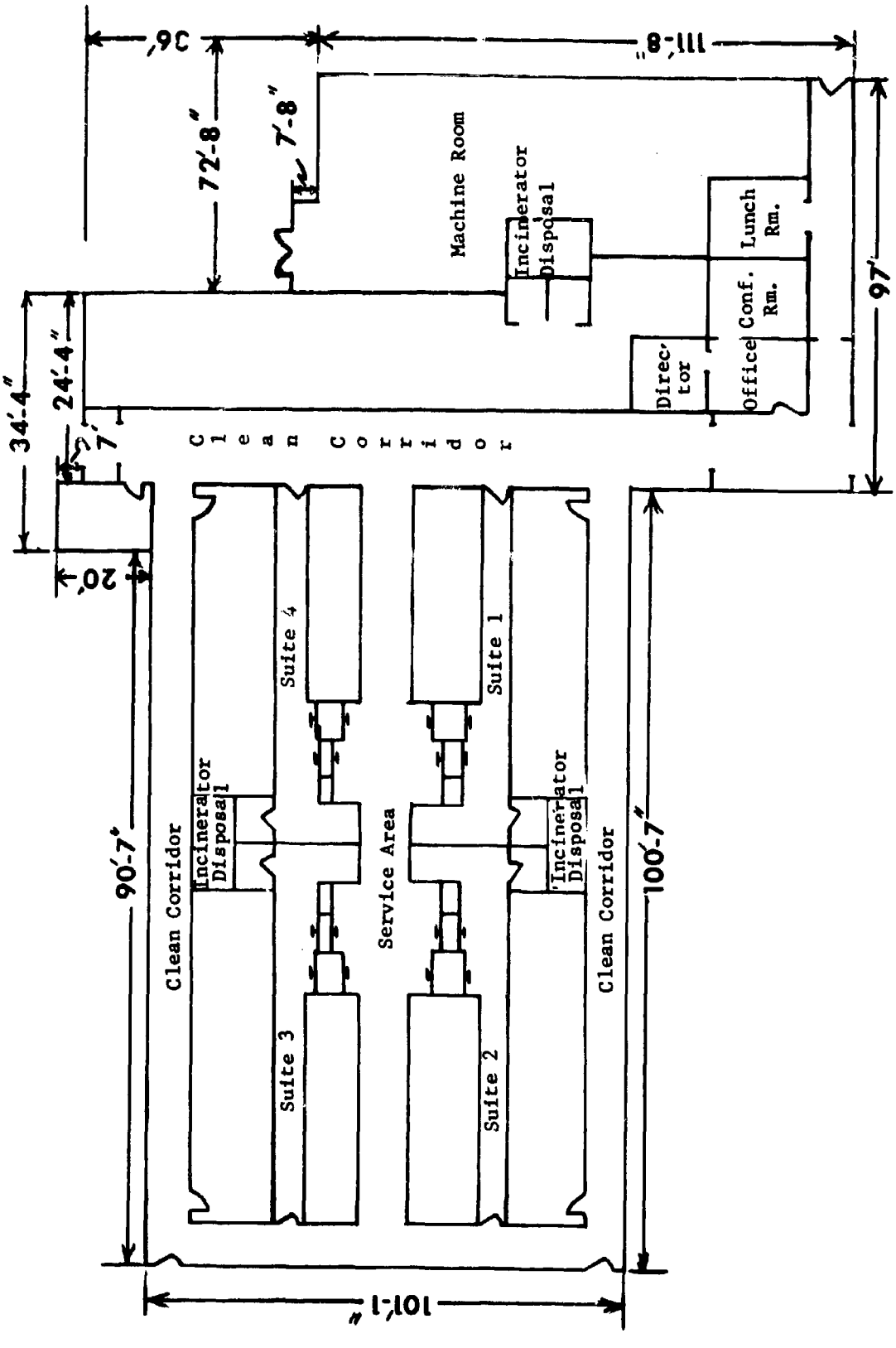

Figure 3. General Floor Plan.

Figure 4. Laboratory Viewing Window.

Figure 5. Ultraviolet Pass Box, Intercommunication System.

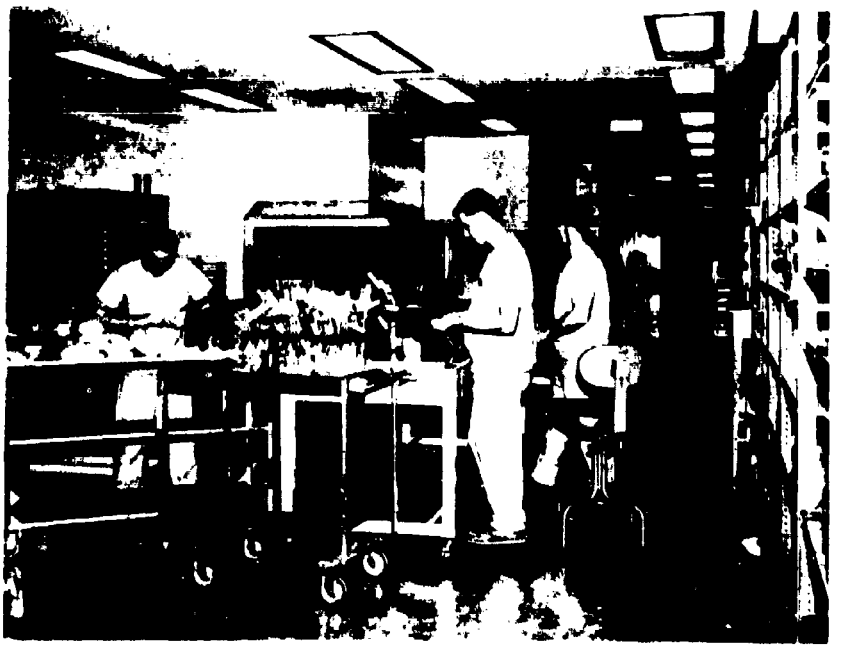

Figure 6. Central Service Area.

Figure 7. Wall-Mounted Service Control Panel.

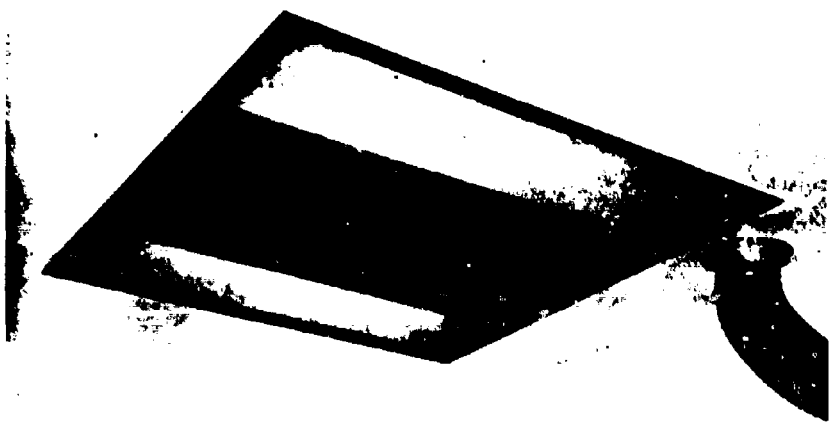

Figure 8. Supply Ventilation in Ceiling with Glass Panels for Fluorescent Lights.

Figure 9. Fluorescent Light Serviceable from Crawl Space.

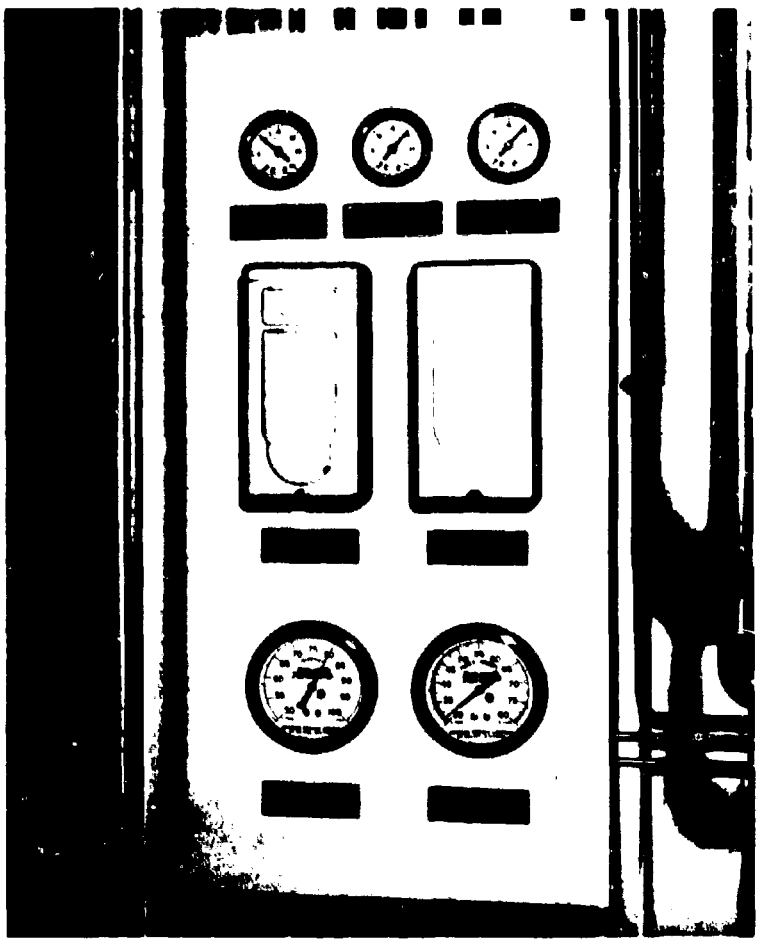

Figure 10. Attic External Controls — Supply Ventilation.

Versatility was designed into the laboratory suites by providing (i) connections for additional hoods; (ii) hoods mounted on rollers for relocation within the same room, suite, or other suites; (iii) removable panel walls for enlargement of rooms or to connect two suites; (iv) dual exhaust filter plenums to permit continuous operation; (v) individual supply and exhaust systems for each suite; (vi) quick-disconnect couplings for services to the laboratory; (vii) a refuse incinerator in each suite; and (viii) central control of all blowers and major mechanical equipment (Fig. 11).

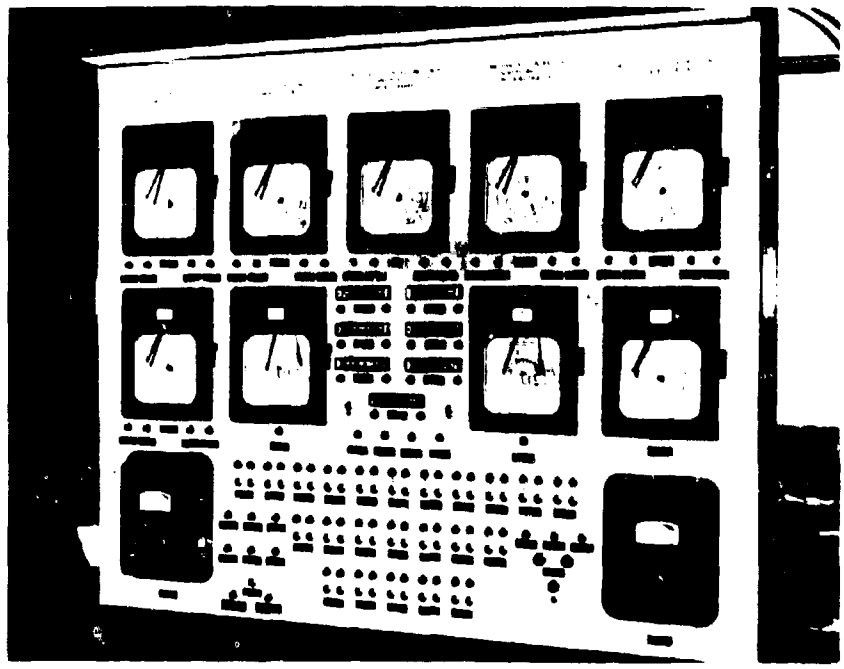

Figure 11. Building Master Control Panel.

The purpose of the biological safety testing program was fourfold; (i) to demonstrate the hazards of certain biological operations; [1] (ii) to determine any hazards associated with equipment operation; (iii) to determine if the equipment, as designed, was functioning properly; and (iv) to determine if operation of the building presented biological hazards to personnel or to the surrounding community.

In the design of the biological safety tests a few basic concepts must be considered and technical validity assessed accordingly. Challenge doses of test microorganisms exceeded the estimated normal operating amount. They were adjusted to permit recovery of microorganisms so that comparisons could be made, and to demonstrate the various hazards. Some biological testing was expedited because of the time allotted (one week). Similar pieces of equipment had to be tested without the benefit of the results of previous tests. Therefore, some tests appear unrealistic or information is questionable and the tests should be repeated later.

The author's mention of a brand name is not to be construed as an endorsement of the product, but is used to characterize the type of equipment.

Controls were run on all sampling media and dilution blanks, and were found satisfactory.

II. SAFETY CABINETS

A. DESCRIPTION

The biological safety cabinets throughout the building are standard six-foot gas-tight stainless steel cabinets manufactured by Kewaunee* (Fig. 12). Each cabinet is equipped with one 34-inch cold cathode ultraviolet lamp (782-L-30, 17 watt), three fluorescent lamps, and quick-coupler connections for gas, air, electrical, and vacuum service. However, none of the cabinets is equipped with hot or cold water or with a drain. Each cabinet is exhausted through a filter pocket, mounted directly behind the cabinet (Fig. 13), that contains two layers of fiber glass filter medium,** 2 feet by 3 feet by ½ inch (50 FG). The filter connects by a section of flexible fiber glass duct (Fig. 12) to the building exhaust duct system that terminates in a plenum containing the building exhaust filters. A manually controlled damper to adjust cabinet air flow is located between the flexible hose and the rigid pipe on the exhaust side of the filter. The cabinets are mounted on rollers for relocation within the room, suite, or other suites according to working requirements. A magnehelic gauge on top of the cabinet indicates the negative pressure within the gloved cabinet, and a second gauge is installed across the filter to indicate the increasing resistance as the fiber glass filter medium becomes dirty and needs changing. Two absolute filters (8 by 8 by 6 inches) on each end of the cabinet provide the necessary makeup air when the hinged cabinet front is closed and gloves are attached. The cabinet is equipped with two pairs of 5-inch glove ports for use with arm-length neoprene gloves. The hinged cabinet viewing window is made of acrylic plastic (Fig. 12).

All 11 cabinet filters were evaluated with a suspension of Serratia marcescens organisms to determine the efficiency of the filters on the back of each cabinet, to determine the travel distance of the microorganisms along the duct-work, and to determine the efficiency of each suite's main plenum (equipped with pockets of 50 FG fiber glass filter medium). Modifications to show and evaluate biological hazards of working with infectious microorganisms were incorporated in the standardized cabinet testing technique, and where appropriate will be noted in the text.

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* Kewaunee Manufacturing Co., Adrian, Michigan.

** Filter medium is composed of superfine fibers of spunglass wool averaging 1.28 microns or less in diameter bonded with a phenolic binder. It is capable of withstanding 600 F and 100% relative humidity.

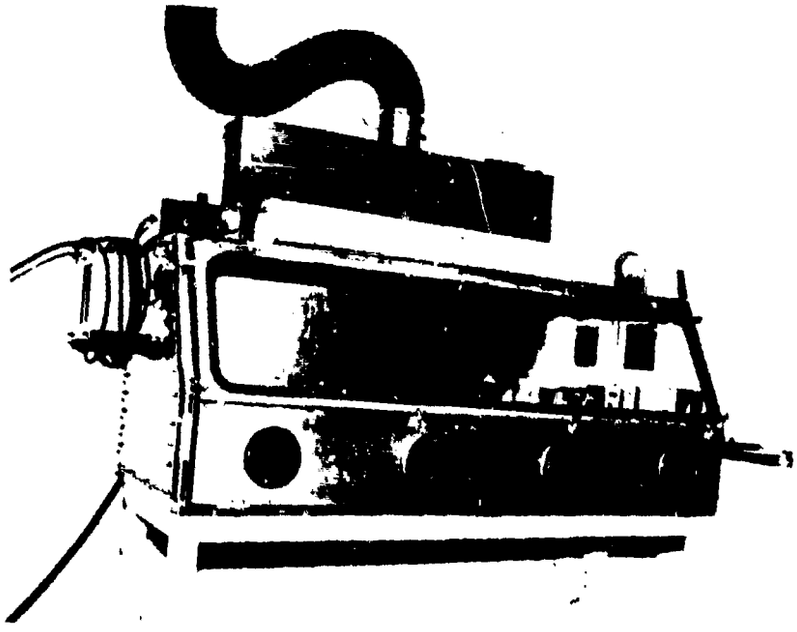

Figure 12. Kewaunee Biological Cabinet.

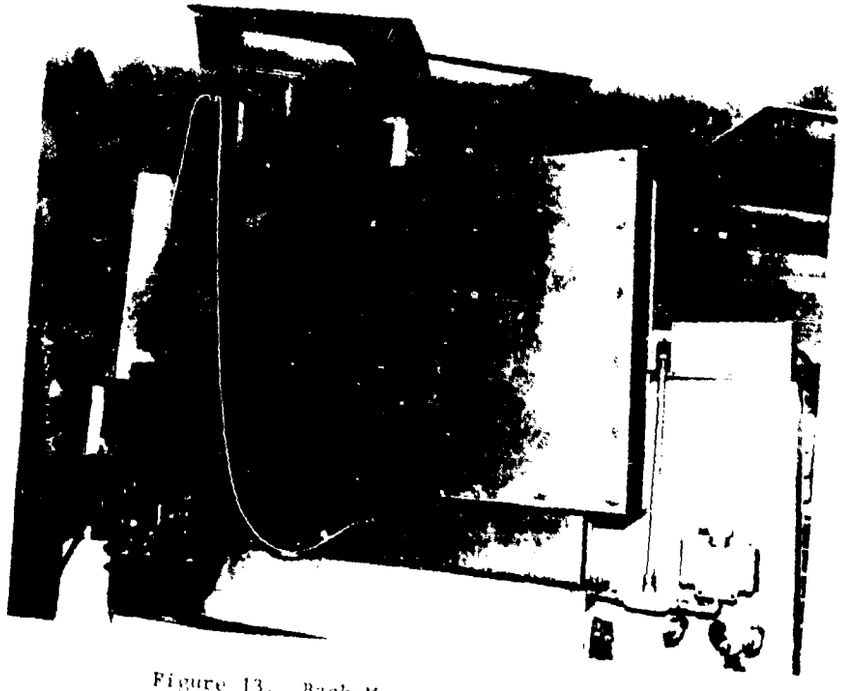

Figure 13. Back-Mounted Exhaust Filter.

B. TESTING CABINET FILTERS

A liquid suspension of S. marcescens microorganisms was prepared from the stock culture (count 1.5×10^{10} organisms per ml). The manually controlled damper was set to give a reading on the Dwyer gauge of 2.8×10^{3} linear feet per minute. With this setting the air flow across the open-front cabinet as checked with an Alnor Velometer was a minimum of 55 linear feet per minute. At this setting the theoretical cabinet exhaust rate as designed is 250 cubic feet per minute (cfm). This design figure was used throughout the cabinet safety testing to obtain the theoretical challenge concentration.* The calculated theoretical concentration of S. marcescens organisms used was 5.5×10^{5} organisms per cubic foot of air. This was obtained by diluting the stock culture in sterile distilled water to a count of 1.5×10^{7} organisms per milliliter immediately before dissemination.

The adjusted liquid culture was disseminated for ten minutes into the cabinet with a pneumatic atomizing nozzle (see the Appendix for description) to provide a dense cloud of the test microorganisms.

Bacterial aerosol sampling locations were (i) immediately above the cabinet filter (Fig. 14), (ii) at mid-point along exhaust duct between cabinet filter and suite's exhaust plenum, (iii) on contaminated side of exhaust filter plenum, and (iv) on clean side of exhaust filter plenum (Figs. 15 and 16). The air within the enclosed exhaust system, which was originally hydrostatically tested at 8 inches of water pressure, was sampled by inserting a short section of ¼-inch OD copper tubing (gradual bend into air stream) into the duct and then attaching a sieve air sampler[2] with a ¼-inch ID heavy-walled rubber tubing. The sieve sampler in turn was connected to a Gast portable exhaust pump. This sampling arrangement permitted one cfm of exhaust air from within the duct to be drawn across the surface of the culture medium plates in the sampler.

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* Sample Calculations:

1. Organisms per cubic foot of air.

$$\frac{\text{ml of culture disseminated per minute} \times \text{organisms per ml}}{\text{air exchange (cfm)}} =$$

organisms per cubic foot (challenge concentration)

2. Per cent efficiency of fiber glass filters.

$$\text{per cent efficiency} = \frac{(\text{number in}) - (\text{number out})}{(\text{number in})} \times 100$$

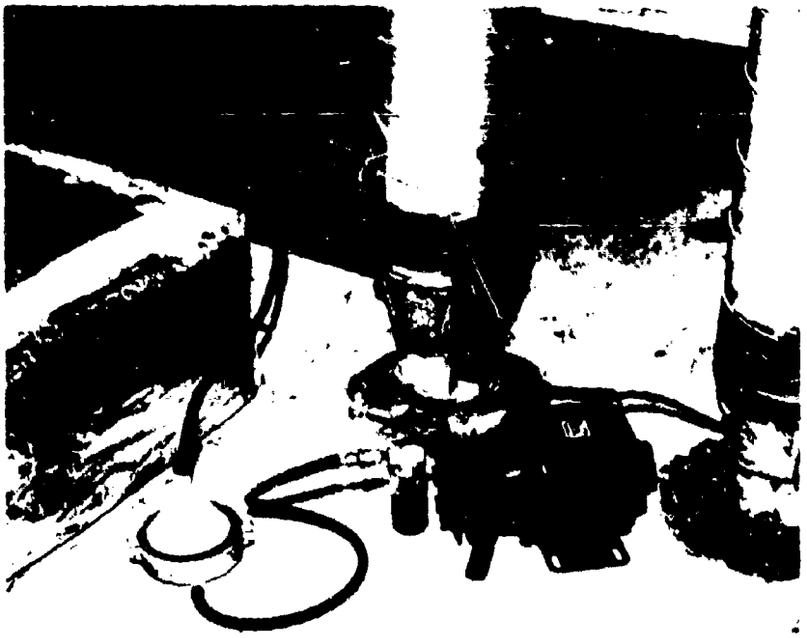

Figure 14. Aerosol Sampling Immediately above Cabinet Filter.

Figure 15. Air Sieve Sampling from Clean and Hot Sides of Filter Plenum.

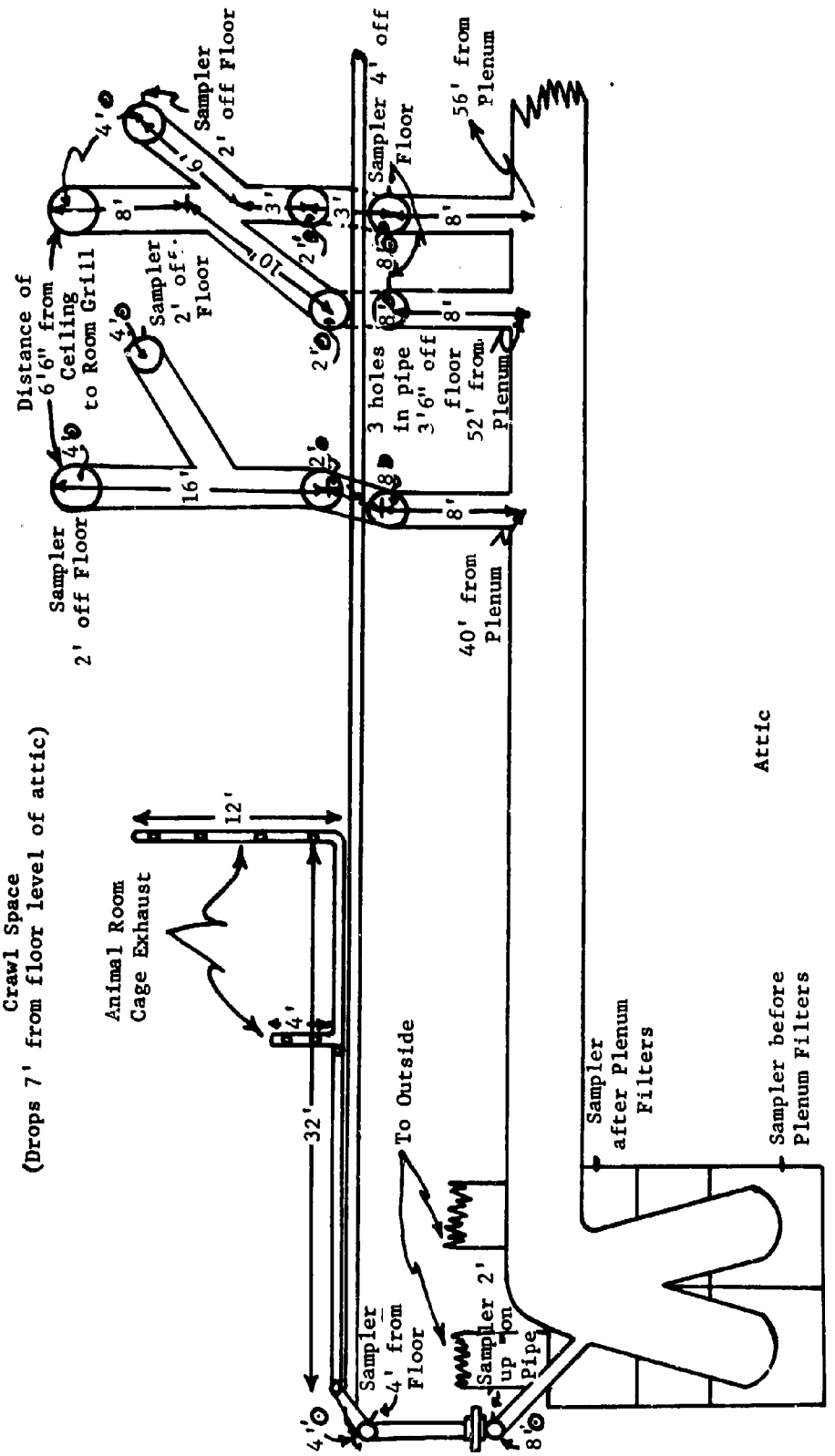

Figure 16. Typical Contaminated Exhaust Layout (Crawl Space) for Suite.

Before testing each cabinet filter in Suite 4, a 10-minute control sample was taken for the test organism (time period A). Then a 10-minute sample was drawn during dissemination of the test organism (time period B). Finally, two successive 10-minute samples were drawn after dissemination (time periods C and D). Modifications were made during tests of cabinet filters in three suites to expedite completion of the tests as follows: the culture was disseminated for 5 minutes instead of 10, the control sample was taken for 5 minutes (time period A), then a 1-minute sample was taken (time period B), followed by a 4-minute sample (time period C), and subsequently a 10-minute sample post-dissemination. All sieve samplers were sterilized before each use by autoclaving or heating the sampler cover with an alcohol flame. When slit samplers were used ethyl alcohol was poured through the slit opening and ignited.

C. RESULTS AND ANALYSIS

Results of all tests of safety cabinet filter exhausts are shown in Table 1. The data show that:

1) Test organisms were recovered on the exhaust side of the cabinet filter. This was anticipated because of the large concentration of microorganisms used during all testing. Normally, one would not expect to have as dense an aerosol cloud created during normal operating conditions.

2) Organisms were recovered inside the exhaust duct as far as the exhaust filters.

3) Actual recovery of organisms may be lower than expected because of (i) loss in viability of the organisms due to the physical forces exerted by the atomizing device, (ii) loss of organisms due to impingement along the duct system and along the copper or rubber sampling tubes, (iii) efficiency of the samplers used for collecting the organisms, (iv) probable existence of a heterogeneous cloud rather than a homogeneous cloud of organisms throughout the exhaust ventilation system, and (v) normal decay rate of aerosolized S. marcescens.

4) The relatively high recovery of organisms at time period D, Suite 4, Room B can be attributed to temporary partial clogging of the pneumatic atomizing nozzle so that the total volume of microorganisms was not atomized in the normal 10-minute dissemination time. Total time for dissemination in this test was 15 minutes; therefore, with the extended dissemination time, one would expect proportional recovery of organisms in greater concentrations at a later sampling time.

5) The cabinet exhaust filters are removing microorganisms in proportion to the type of filter medium used. This shows that the filters were properly installed and filter frames do not have large openings.

TABLE 1. RECOVERY OF S. MARCESCENS IN TESTS OF BIOLOGICAL SAFETY CABINET FILTERS

Suite and Room	Time Period	Average Organisms Per Cu. Ft. at Sieve Sampler Location 1 Just Above Cab. Filter	2 Midpoint in Duct From 1 to 3	3 Contam. Side Exhaust Filter	4 Clean Side Filter	Per Cent Efficiency[a] Exhaust Filter
4-B	A[b]	Neg.	Neg.	Neg.	Neg.	
	B	TNTC[c]	6.5	15.7	Neg.	100
	C	TNTC	24.8	12.9	Neg.	
	D	TNTC	17.9	8.4	Neg.	
4-C	A	Neg.	Neg.	1[d]	Neg.	
	B	TNTC	TNTC	TNTC	Neg.	100
	C	TNTC	16.6	0.4	Neg.	
	D	Neg.	Neg.	Neg.	Neg.	
1-B	A	Neg.	For remainder of tests midpoint sampling location was eliminated because organisms traversed the duct and location 3 gave similar information.	Neg.	Neg.	
	B	29		196	Neg.	99.999
See text for deviation.	C	70		TNTC	0.5	
	D	21.3		1.2	Neg.	
1-E	A	Neg.		Neg.	Neg.	
	B	Neg.		3	1	99.999
	C	Neg.		2	0.5	
	D	Neg.		Neg.	Neg.	
1-C	A	Neg.		Neg.	Neg.	
	B	227		No impingement	1	99.999
	C	TNTC		No impingement	1.7	
	D	TNTC		No impingement	0.1	
3-E	A	Neg.		Neg.	Neg.	
	B	Neg.		91	Neg.	100
	C	2.5		98.2	Neg.	
	D	0.2		0.7	Neg.	
2-E	A	Neg.		Neg.	Neg.	
	B	349		5	Neg.	100
	C	108		5.7	Neg.	
	D	Neg.		Neg.	Neg.	
2-D	A	Neg.		Neg.	Neg.	
	B	402		65	Neg.	100
	C	81		45	Neg.	
	D	Neg.		0.2	Neg.	
2-C	A	Neg.		Neg.	Neg.	
	B	1		Neg.	Neg.	100
	C	70		28	Neg.	
	D	3.2		1.7	Neg.	
2-B	A	Neg.		Neg.	Neg.	
	B	177		12	Neg.	99.999
	C	84		21	0.25	
	D	3.8		0.7	Neg.	
3-B	A	Neg.		Neg.	Neg.	
	B	297		Neg.	18	99.997
	C	90.7		0.7	24	
	D	7		Neg.	0.9	

a. See text for sample calculation. Efficiency of exhaust plenum filter obtained by using average counts of time periods B, C, and D recovered on both the clean and contaminated side of filters from sampler locations 3 and 4.

b. Time periods: A, control, B, dissemination, C and D, post-dissemination.

c. TNTC: too numerous to count; where TNTC is shown in data, counts of 5.5×10^{4} org/ft^3 were substituted (the theoretical concentration).

d. Recovery explained by using same location for sampling within 30 minutes after Text 2. Probably caused by flaking of organism from duct wall.

III. MICROORGANISMS TRAVERSING EXHAUST DUCT

A. DESCRIPTION

The individual cabinet exhaust filter was removed in Suite 4, Room B, to determine if microorganisms traverse the entire length of the exhaust duct and reach the main suite's exhaust filters. The front panel and gloves were installed on the cabinet before the test microorganisms were disseminated.

B. TESTING

Sieve samplers were located at the 4 sampling locations described in Section II, B. Control samples, each representing 10 minutes' sampling, were taken at each sieve sampler location. The Dwyer gauge showed a differential of 2.8×10^3 linear feet per minute.

The S. marcescens culture was disseminated with the pneumatic atomizing nozzle for 10 minutes. Theoretical concentration was 5.5×10^6 organisms per cubic foot.

C. RESULTS AND ANALYSIS

The sieve sampler plates showed heavy contamination (TNTC) immediately above the cabinet filter, at the midpoint along the duct (between cabinet filter and suite's main exhaust plenum), and on the contaminated side of the filters, but considerable reduction on the clean side of the filters. The theoretical concentration of 5.5×10^6 organisms per cubic foot was reduced to two organisms per cubic foot on the clean side of the filter, a reduction of 99.999%. This reduction may be biased because of decreased viability, etc. as described in Section II, C,3. However, in spite of uncontrollable variables, all indications were that the filter performed satisfactorily.

The data showed that the test organisms were traversing the entire length of the duct system and being filtered out of the exhaust air by the plenum filters, and that the exhaust blower was operating satisfactorily.

IV. AIR EXHAUST SYSTEMS

A. ANIMAL CAGES

1. Description

The exhaust tube carries contaminated air from sealed animal cages to the plenum. A liquid suspension of S. marcescens organisms was disseminated into one (location 10, Fig. 17) of the exhaust tubes for animal cages (Fig. 18) in Suite 4, Room C, and in Suite 3, Room E. The objectives of these tests were to determine (i) if cross contamination occurs between adjacent tubes of the ventilation system, (ii) if the booster exhaust blower in the attic (Fig. 19) is producing an aerosol and thereby allowing microorganisms to escape to the attic from the pressure side of the blower, (iii) if organisms traverse the length of the duct system, and (iv) the hazard associated with the absence of a filter before the cage exhaust system enters the main filters of the suites.

2. Testing

Samplers were located in the attic along the cage ventilation duct system at (i) the negative side of the booster blower for animal racks, (ii) the pressure side of the blower, (iii) the contaminated side of the filters, and (iv) the clean side of the filters. Sieve samplers were used to collect organisms at stations 1 to 4, and 6 to 9 (Fig. 17) inclusive. At station 5 a one-hour slit sampler[3] was placed beneath the booster blower to assess the hazard, if any, associated with the blower. Stations 6 to 9 inclusive were equipped with sieve samplers to detect cross contamination among the various cage ventilation trunk legs. Should cross contamination occur, the use of ventilated cages for housing experimentally infected animals could invalidate experimental data. The air flow through each cage ventilation trunk leg was 118 cubic feet per minute. The theoretical concentration of test microorganisms disseminated was 1.2×10^7 organisms per cubic foot.

Control samples (10 minutes each) were taken at sampling locations 1 to 5 inclusive, but not at stations 6 to 9 inclusive because (i) the test organism was not used in the area of Room E, (ii) difficulty was experienced in attaching samplers to the ventilation duct system, and (iii) the sampling plates could be contaminated when the disseminating device was attached to the one leg of the ventilation system.

The organisms were disseminated with the pneumatic atomizing nozzle and samples were taken at all locations for 10 minutes during dissemination. Two 10-minute samples each were taken following dissemination at stations 1 to 4 inclusive. The slit sampler was operated for one hour.

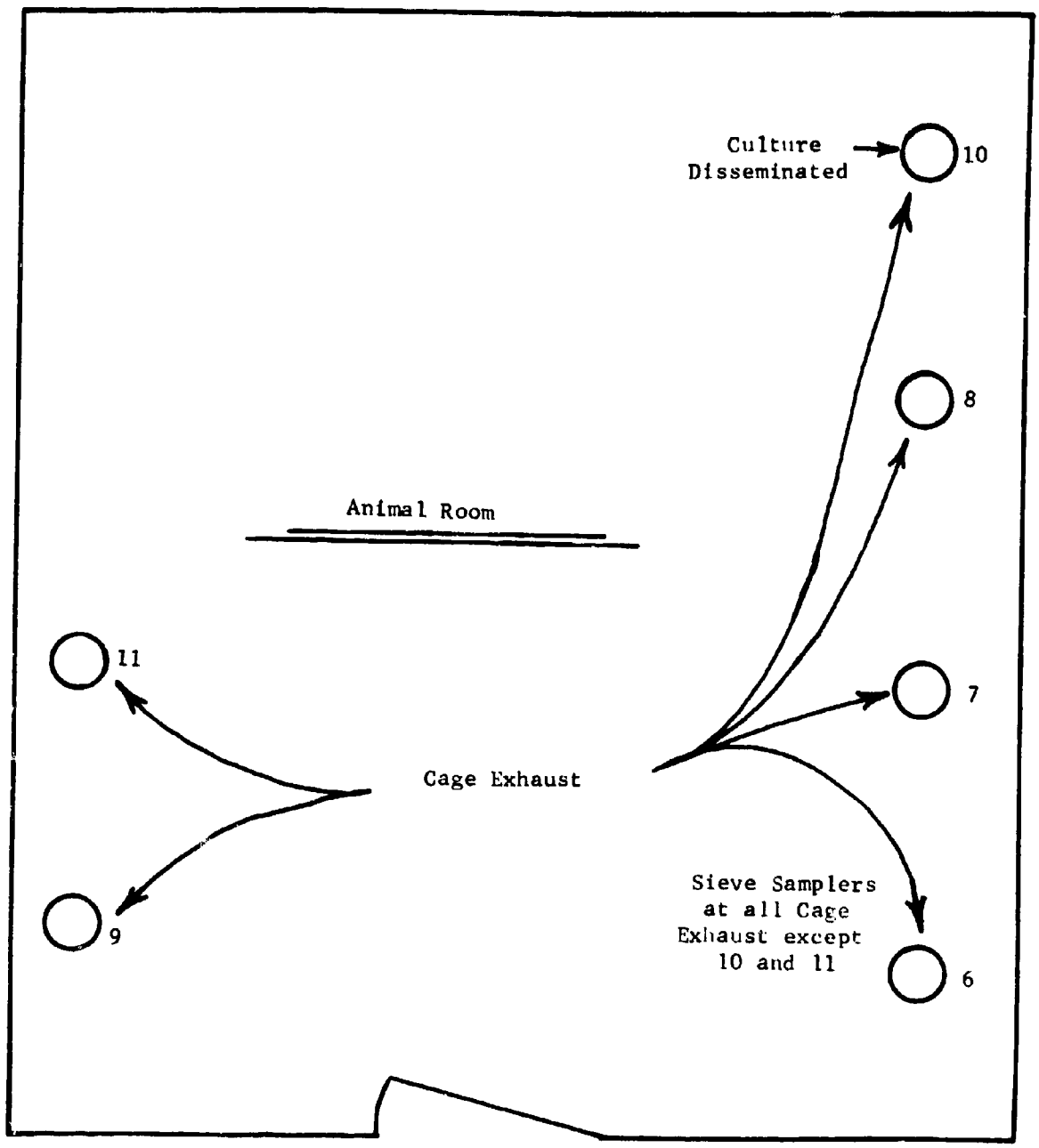

Figure 17. Animal Room Exhaust System Sampler Locations.

Figure 18. Animal Cage Ventilation Trunk Legs.

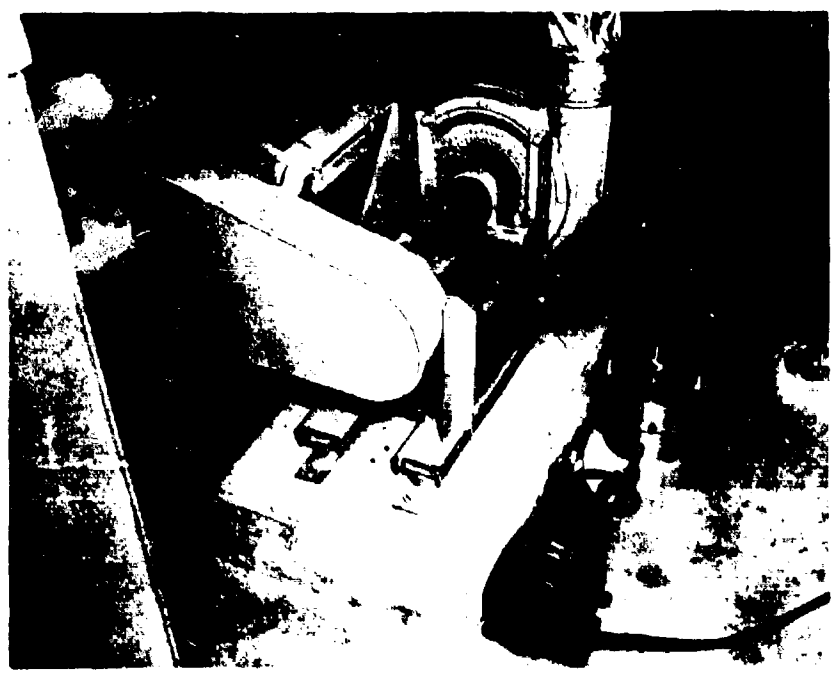

Figure 19. Animal Exhaust - Ventilated Cage Blower.

In testing Suite 3, Room E, two sieve sampling locations, one on the negative and one on the positive side of the booster blower, were not sampled to avoid contamination of the attic by spill-over of organisms from the Gast pump sampler exhaust system.* In addition, the quantity of organisms disseminated was decreased to 1.2×10^6 organisms per cubic foot to permit assay as per cent reduction on the exhaust filter. Otherwise, all testing was done in the same manner as that in Suite 4, Room E.

3. Results

The data in Table 2, obtained from these tests, indicate that: (i) cross contamination does not occur between adjacent legs of the animal exhaust ventilation system, (ii) the booster blower in Suite 3 is not producing a measurable aerosol in the attic, (iii) the test microorganisms are traversing the entire length of the ventilation system and reaching the filters (plenum), (iv) considerable hazard would exist if the suite's exhaust filters were to become defective and cause escape of organisms either to the atmosphere or to the attic itself, and (v) reduction of organisms in Suite 3 by the exhaust plenum filters was 99.999% efficient.

Recovery of one colony of S. marcescens at station 7 (see Table 2, Suite 4, Room E) may have been due to external contamination, because the same person disseminated the culture and immediately afterward collected that sampling plate.

Recovery of one colony of S. marcescens at the control stage (see Table 2, Suite 4, Room E) at station 5 (one-hour slit sampler) may be attributed to an aberrancy in operating the slit sampler, i.e., the distance between the slit and surface of the agar is such that when organisms in heavy concentrations enter the orifice they are not immediately impinged, but remain suspended in the air space within the sampler and later settle on the agar surface.

4. Analysis

Because organisms recovered on the slit sampler indicated aerosol escape from the booster blower serving Suite 4, Room E, the blowers in Suites 2 and 4 (repeated test) were tested without other additional sampling.

S. marcescens was disseminated at a concentration of 1.2×10^7 organisms per cubic foot as in the previous test.

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* See explanation in Section IV, A, 4.

TABLE 2. RECOVERY OF S. MARCESCENS FROM ANIMAL EXHAUST VENTILATION SYSTEM

Sampler Location (Section IV, A, 2)	Average Microorganisms Recovered Per Cubic Foot: Control	Dissemination	Post-Dissem. 1-10 Minutes	Post-Dissem. 10-20 Minutes
		Suite 4, Room E		
1 Negative side blower	neg.	TNTC	TNTC	15.5
2 Pressure side blower	neg.	TNTC	TNTC	2.7
3 Contam. side filter	neg.	TNTC	TNTC	neg.
4 Clean side filter	neg.	0.9	9.9	neg.
5 Slit beneath blower - see under Sampling Period below				
6 Trunk leg	ND[a]	neg.	ND	ND
7 Trunk leg	ND	0.1	ND	ND
8 Trunk leg	ND	neg.	ND	ND
9 Trunk leg	ND	neg.	ND	ND
		Suite 3, Room E		
3[b] Contam. side filter	neg.	11	0.2	neg.
4 Clean side filter	neg.	0.2	neg.	neg.
5 Slit beneath blower - negative through entire hour				
6 Trunk leg	ND	neg.	ND	ND
7 Trunk leg	ND	neg.	ND	ND
8 Trunk leg	ND	neg.	ND	ND
9 Trunk leg	ND	neg.	ND	ND
	Sampling Period (minutes)	10-20	20-40	40-60
5 (slit)	0.1	6.8	0.1	neg

a. Not done - see text.
b. Stations 1 and 2 eliminated in this test. See text. Efficiency of filters was calculated at 99.999%.

A one-hour slit sampler was located immediately beneath the booster blower for animal exhaust ventilation in Suites 2 and 4 (retest). Controls were taken for 10 minutes and the slit sampler was operated for one hour. Microorganisms were not recovered in either of these tests, indicating that the booster blowers to the animal exhaust ventilation systems were functioning satisfactorily.

Unexplained recovery of microorganisms on Test 1 of Suite 4, station 5, was investigated further. The author's opinion that microorganisms may spill over through the Gast pump sampler exhaust system was evaluated under controlled laboratory conditions. Tests showed that whenever large quantities of microorganisms (TNTC) are pulled across a sampling plate a certain percentage pass through the pump serving the air sampler and are discharged to the atmosphere. Because aerosol samples were taken on both the negative and positive sides of the cage exhaust blower in Test 1 and the concentration of microorganisms was 1.2×10^7 organisms per cubic foot, spill-over did occur. Therefore, it is concluded that microorganisms recovered at station 5 were those discharged by the other two air sampler Gast pumps, and not from the cage exhaust blower.

5. Discussion

Air exhausted from a cage containing infected animals may contain a high concentration of microorganisms either from aerosolization of microorganisms from the animal's coat (if aerosol-exposed or injected) or from excreta. Therefore, the animal cage exhaust line is considered one of the more hazardous areas within the entire ventilation system. Once the microorganisms reach the pressure side of the blower, the hazard increases accordingly. In view of this, an attempt should be made to reduce the concentration of microorganisms before they reach the pressure side of the blower by inserting an in-line filter between the animal cages and exhaust blower. One suitable type of filter is the Dollinger,* which is manufactured in capacities from 40 to more than 250 cfm.

The hazard to personnel may be further minimized if the line from the exhaust blower to the building plenum is kept at a negative pressure by the main building exhaust blower.

B. RUPTURED EXHAUST DUCT FROM A LABORATORY ROOM

1. Description

To simulate a ruptured duct, three holes (7/8 inch in diameter) were cut in the attic portion of the exhaust duct from Room B, Suite 4.

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* Dollinger Corporation, 11 Centre Park, Rochester, N.Y.

The objective of this test was to simulate and assess the hazards due to parting of the duct seams caused by an accidental blow or by expansion and contraction of the duct. Figures 20 and 21 show sieve and slit sampler locations and placement of holes.

2. Testing

The air within the exhaust duct was sampled in the same manner as previously. Three slit samplers were positioned around the simulated rupture at varying heights (Fig. 20), and sieve samples were taken on both the clean and contaminated side of the exhaust filters. The exhaust rate for this room was 1050 cfm.

Following control sampling, the diluted culture (1.3×10^6 microorganisms per cubic foot) was disseminated for 10 minutes with the pneumatic atomizing nozzle 3 inches in front of the roughing exhaust prefilter (Figs. 20 and 22). Figure 16 shows the exhaust duct layout. The roughing prefilter (coarse glass fiber dust stop, Fig. 22) located within the laboratory room was in place during this test.

The slit samplers were run for one hour. The sieve samples were taken in three 10-minute increments during and after dissemination.

3. Results and Analysis

The data (Table 3) show that organisms will escape from a ruptured duct in spite of the negative pressure on the exhaust duct created by the main exhaust fan for the suite. This escape can be attributed to the turbulence that is created at a rupture site and at the duct bend. The bend nearest to the simulated rupture point was 5 to 6 feet away.

The failure to recover S. marcescens organisms with the slit sampler located above the simulated rupture points indicates that organisms escaping from the duct settle to the floor by gravitational pull.

The main exhaust filter is 99.998% efficient.

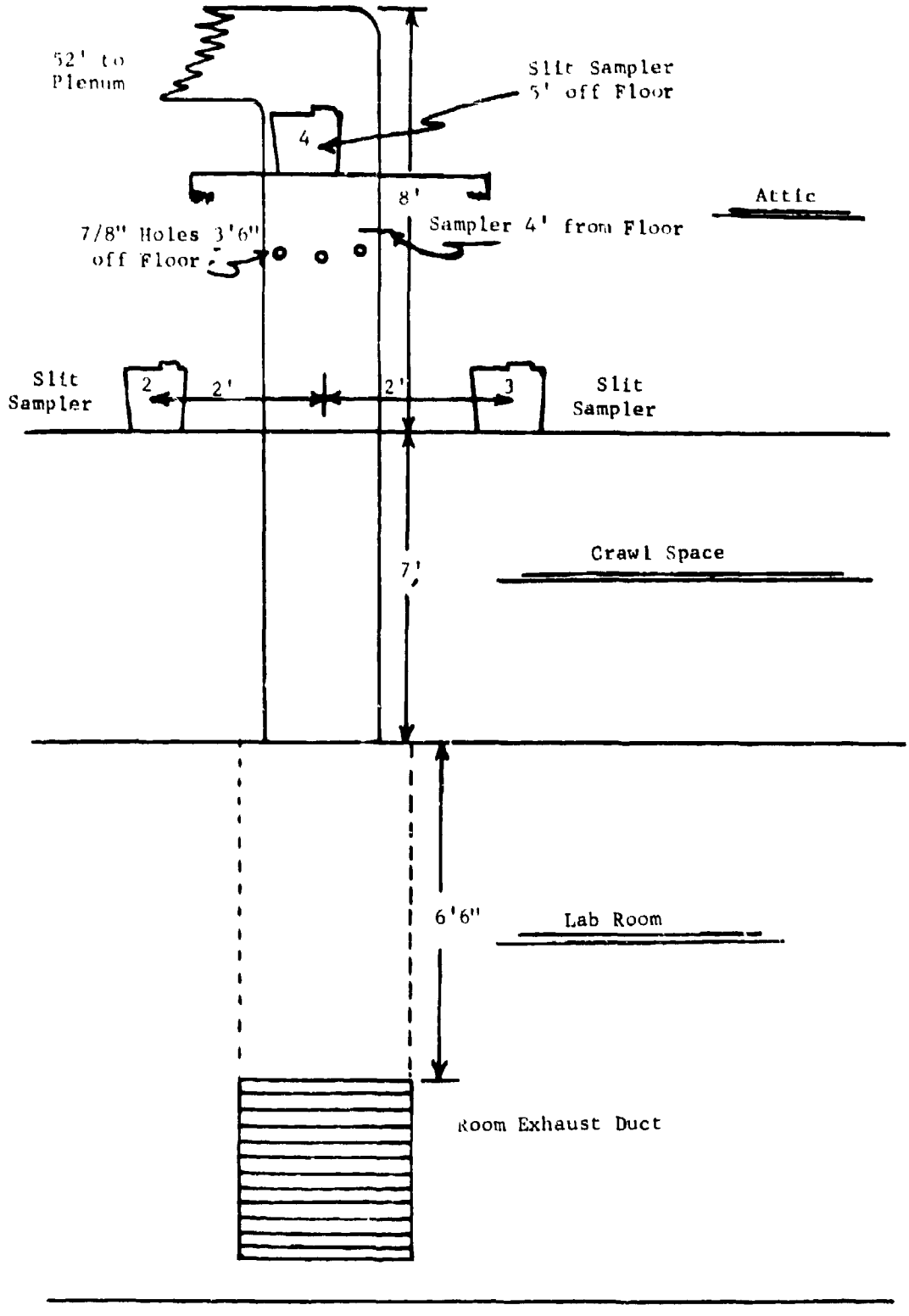

Figure 20. Laboratory Room Exhaust Duct and Sampler Locations.

Figure 21. Simulated Duct Rupture and Sampling Adapter.

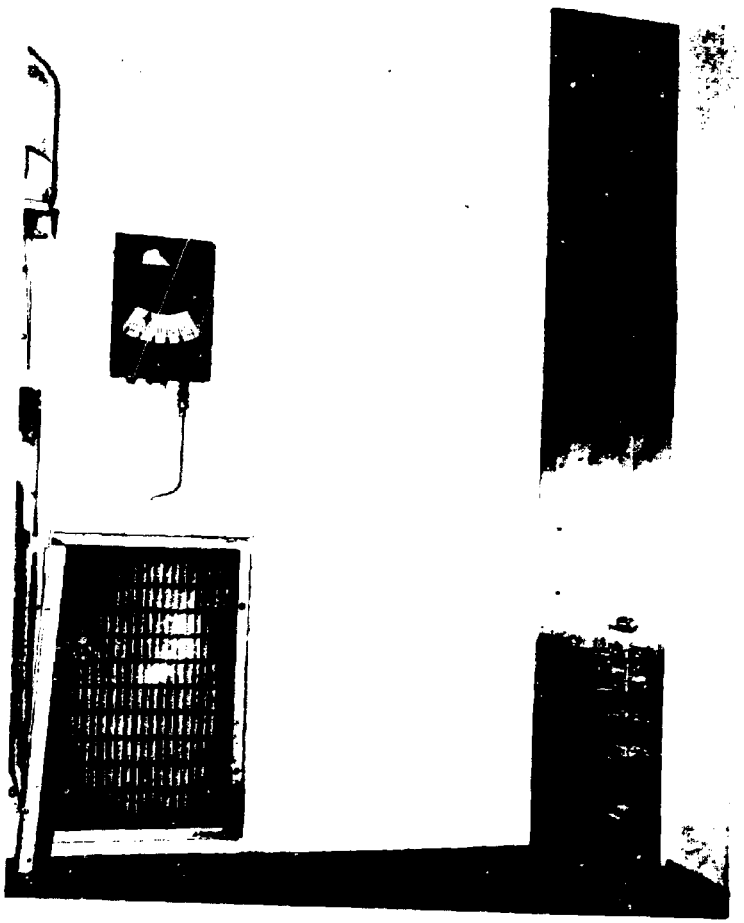

Figure 22. Roughing Exhaust Prefilter and Thru-Wall Service Connections.

TABLE 3. RECOVERY OF S. MARCESCENS FROM SIMULATED EXHAUST DUCT RUPTURE

Sampling Location	Average Microorganisms Recovered per Cubic Foot			
	Control	Dissemination	Post-Dissem. 1-10 Minutes	Post-Dissem. 10-20 Minutes
Sieve (1) midpoint on duct	neg.	TNTC[a]	2.4	neg.
Slit 2 Floor left	neg.	4.8	4.8	4.8
Slit 3 Floor right	neg.	28	28	neg.
Slit 4 5 feet above floor	neg.	neg.	neg.	neg.
Sieve (5) dirty side of filter	neg.	TNTC	neg.	neg.
Sieve (6) clean side of filter	neg.	14.4	neg.	neg.

a. TNTC - too numerous to count.

C. PLENUMS

1. Description

Each suite has a duplicate exhaust filter system complete with blower (Fig. 23) into which all exhaust air from the individual laboratories and cabinets is filtered before being discharged to the atmosphere. Each plenum is equipped with deep-bed, pocket-type fiber glass filter material through which the air passes.

2. Testing

The efficiency of all exhaust filter plenums (including the reserve filters) was determined by disseminating a liquid suspension of S. marcescens. The aerosol was generated by a Challenger sprayer that produces particles ranging from less than 1 to greater than 10 microns in diameter at 50% RH. Forty-two per cent of the particles were less than 6 microns in diameter (see the Appendix). The generator containing the S. marcescens organisms was placed within the contaminated side of the plenum. Control counts

Figure 23. Duplicate Plenum Exhaust Blowers and Valves.

were taken on both the contaminated and clean side of the filters (Fig. 24) with sieve samplers in the same locations used previously. The organisms were disseminated for 10 minutes. After aerosolizing for 2 minutes, a 1-minute control sample was taken at each sampling location. This sampling time delay permitted the aerosol to attain homogeneity throughout the plenum. Subsequently, one 4-minute, and two 10-minute samples were taken on each side of the filter. Data on air flows, filtration efficiency, and organisms recovered during testing are shown in Table 4.

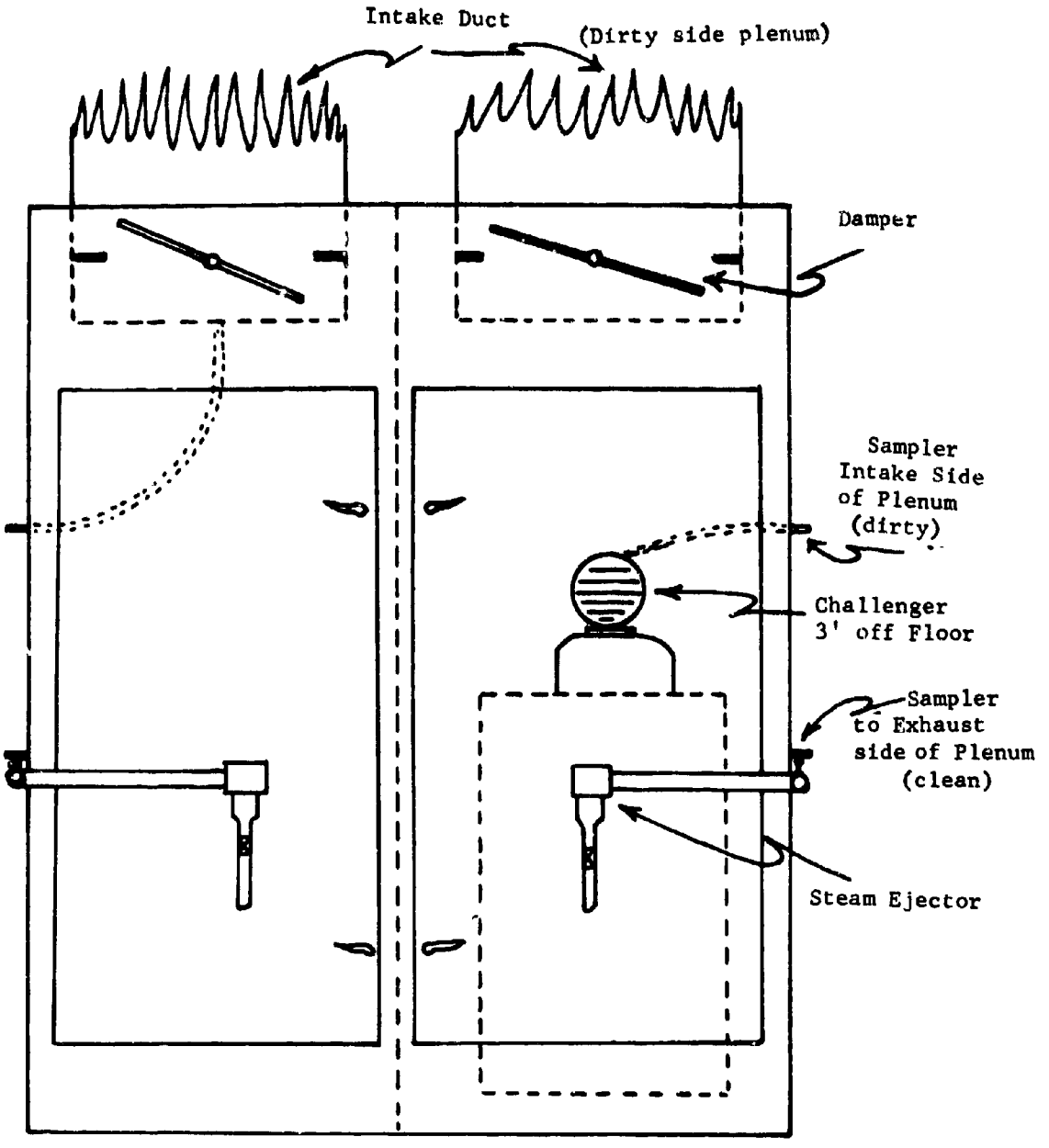

Figure 24. Exhaust Plenum Sampler Locations.

TABLE 4. RECOVERY OF S. MARCESCENS FROM PLENUMS

Filter Plenum Location	Air Flow, cfm	Aerosol Concentration, org/cu. ft.	Sieve Sampler Location	Average Microorganism Per Cubic Foot					Per Cent Efficiency of Filters[c]
				Control	1 Min	4 Min	1st 10 Min	2nd 10 Min	
Suite 1	4920	5.3×10^5	Dirty side	Neg.	Neg.	Neg.	0.5	ND[a]	100
			Clean side	Neg.	Neg.	Neg.	Neg.	ND	
Suite 1-A[b]	4920	5.3×10^5	Dirty	Neg.	263	72	Neg.	ND	100
			Clean	Neg.	Neg.	Neg.	Neg.	ND	
Suite 2	5640	4.6×10^5	Dirty	Neg.	Neg.	Neg.	Neg.	ND	100
			Clean	Neg.	Neg.	Neg.	Neg.	ND	
Suite 2-A	5640	4.6×10^5	Dirty	Neg.	296	90	Neg.	ND	99.996
			Clean	Neg.	23	13	Neg.	ND	
Suite 3	5840	4.5×10^5	Dirty	Neg.	237	49	Neg.	ND	99.999
			Clean	Neg.	5	2.5	Neg.	ND	
Suite 3-A	5840	4.5×10^5	Dirty	Neg.	9	13	Neg.	ND	100
			Clean	Neg.	Neg.	Neg.	Neg.	ND	
Suite 4	6300	4.2×10^5	Dirty	Neg.	20	60	Neg.	ND	99.999
			Clean	Neg.	Neg.	0.25	Neg.	ND	
Suite 4-A	6300	4.2×10^5	Dirty	Neg.	TNTC	TNTC	Neg.	Neg.	99.996
			Clean	Neg.	3	0.25	Neg.	Neg.	

a. Not done.
b. For remainder of plenum tests, the last 10-minute sampling was eliminated.
c. Per cent efficiency determined by using the average of the 1- and 4-minute sampling recovery data from the clean side of the filters, and the challenge concentration.

3. Results

The data show that the filters are at least 99.996% efficient. This compares favorably with the efficiency obtained during testing of the cabinet filters, Section II, C. However, failure to recover organisms in plenum 2 with the sieve samplers can be attributed to defective aerosolization or to improper positioning of the sampling tubes.

Failure to recover larger quantities of organisms in the contaminated side of the plenum may be explained by one or a combination of the following factors: (i) efficiency of sampling device, (ii) positioning of disseminating generator, (iii) positioning of sampling tube in relation to aerosol cloud, or (iv) air flow pattern through the filter plenum. However, an assessment was made from the data available in Table 4.

Each filter plenum is working satisfactorily and can be used to remove microorganisms from the laboratory building exhaust air before the air is discharged to the outside atmosphere.

The results obtained on the plenum filtration of the air in the cabinet testing phase also show that the filters are installed properly and operating at designed efficiency.

A more realistic assessment can be made of filters within plenums through use of a large-volume air sampler and modified techniques in locating disseminator and samplers.

V. SEWAGE PRETREATMENT TANK

A. DESCRIPTION

The sewage system throughout the laboratory building is made of pyrex pipe that terminates in either of two vertical glass-lined steel tanks (9 feet in diameter by 13 feet high). The tanks, properly valved for alternate use, are connected to the drain system and are located in a building, two-thirds of which is below ground level, adjacent to the main laboratory building. The capacity of each tank is 5,000 gallons. Each tank is equipped with a U-shaped 1½-inch OD 16 BWG* tubing to heat the water from approximately 10 to 102 C using steam at 40 psig. A steam jet, located 3 feet above the tank bottom, is constructed from ½-inch 304 stainless steel pipe (standard weight), equipped on the discharge side with a Penberthy No. 351 all-bronze steam muffler and a 12-inch-long S.S. pipe extension outlet. The steam jet is intended to provide a homogeneous mixture and insure sterilization of the effluent waste. The temperature sensing element is located at the center of the tank. Each tank is equipped with a manhole, inlet and outlet connections, temperature and liquid-level recorders, and a vent that discharges through a Dollinger bacterial pipeline filter to the atmosphere. Tanks are designed to operate at 102 C.

Biological tests were conducted to determine the retention time at 102 C necessary to sterilize liquid wastes containing vegetative or spore-forming bacteria.

B. TESTING

1. Test 1

The pretreatment tank was allowed to fill to a height of 6 feet with normal waste containing human fecal material. After adding 3 liters each of a suspension of Serratia marcescens (vegetative cell) and of Bacillus subtilis var. niger (spores), a control sample was taken through the manhole. The microorganisms were added to the pretreatment tank by pouring the cultures into the water closet and flushing. The addition of cultures to the tank is described in Section VI, B, 1.

Prior to addition of effluent waste to the tank, a sampling adapter** was placed on the embossed area of the drain elbow on pretreatment tank 2. The adapter permitted sampling of the effluent waste from the tank during the sterilization cycle.

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* Birmingham wire gauge.

** Fort Detrick Drawing 6-93-1-1757.

After the heating coil and steam mixing jet were turned on, 1¼ hours were required to heat the effluent waste to 102 C. After 40 minutes at that temperature, a sample of the effluent was taken through a 4-inch clean-out plug located approximately 6 feet from the discharge valve. This was done by cracking the 8-inch gate valve near the bottom of the tank and allowing the waste to flow through the discharge line. The technique of taking the first sample prevented contamination of the sampling adapter.

After heating with both the steam jet and the coil for 2½ hours, the temperature in the tank reached 116 C and was held there for 1 3/4 hours. Inasmuch as this temperature was not the desired operating condition, the tank was vented to atmosphere and cold water was added to raise the liquid height to 8 feet and to drop the temperature of the effluent waste to 102 C. It is believed that, with only 6 feet of water and refuse in the tank, the temperature sensing element was intermittently out of the water because of the turbulence created by the 40 psig steam jet. Therefore, the temperature recorded during a part of the test was that of live steam instead of the water. For the remainder of this test the mixing steam jet was turned off. Samples were taken of the effluent waste at 2, 4, and 6 hours through the sampling adapter on the elbow of the discharge line beneath the pretreatment tank. All sampling time periods were calculated from the first time the temperature of the water reached 102 C.

Following the addition of the water and raising the level to 8 feet (total quantity of water 3,000 gallons), the temperature recording device was set at 102 C. However, because of a malfunction of the control device on the steam coil, the temperature for the remainder of the test was 107 C. In spite of the difficulties encountered the test was completed.

Each sample of effluent was treated as follows: One-tenth ml was inoculated on duplicate nutrient agar plates, one ml was inoculated into duplicate tubes containing 10 ml of nutrient broth, and ten ml were inoculated into duplicate tubes containing 25 ml of lactose broth. Duplicate sets were made, one for incubation at 30 C, the other at 37 C. Data obtained from this test are presented in Table 5.

2. Test 2

Before Test 2, a pressure gauge was installed on the exhaust vent line between the pretreatment tank and cut-off valve before the Dollinger in-line vent filter.

TABLE 5. RECOVERY OF MICROORGANISMS FROM PRETREATMENT TANK STERILIZATION CYCLE, TEST 1

Sample			Incubation Temp, 30 C		Incubation Temp, 37 C		
Time	Location	Org.	Nutrient[a] Agar	Nutrient Broth	Lactose Broth	Nutrient Agar	Nutrient Broth
Control	Dip sample from manhole	B. subtilis	TNTC	Positive	Positive & gas	TNTC	Not Done
		S. marcescens	TNTC			106	
40-min	4-inch clean out	B. subtilis	TNTC	Positive	Positive & gas	142	Positive
2 hour	adapter	B. subtilis	Neg.	Neg.	Neg.	Neg.	Neg.
		S. marcescens	Neg.	Neg.	Neg.	Neg.	Neg.
4 hour	adapter	B. subtilis	Neg.	Neg.	Neg.	Neg.	Neg.
		S. marcescens	Neg.	Neg.	Neg.	Neg.	Neg.
6 hour	adapter	B. subtilis	Neg.	Neg.	Neg.	Neg.	Neg.
		S. marcescens	Neg.	Neg.	Neg.	Neg.	Neg.

a. Average of duplicate plates.

Liquid suspensions of both B. subtilis and S. marcescens were added to pretreatment tank 2 in the same manner as in Test 1. The effluent was not drained from the tanks between tests. The culture was flushed down the water closet nearest the pretreatment tanks. A total of 3½ liters of each culture was added to the pretreatment tank that contained 2,959 gallons of water, giving a theoretical concentration of 4.7×10^6 organisms per ml. The temperature of the wa' in the pretreatment tank was 89 C.

Immediately after the cultures were added, the heating coil and steam jet were turned on for 10 minutes to mix the microorganisms with the wastes. Control samples were obtained by taking samples through the adapter sampling port.

This test required 30 minutes to raise the temperature of the liquid in the tank from 89 to 102 C. Subsequently, the steam jet was turned off to prevent overheating as occurred on Test 1. The design operating conditions on the tank called for use of both the coil and steam jet. However, it was decided to determine if the coil could maintain the temperature at 102 C without assistance from the jet. The pressure on the tank maintained at 102 C was 0.75 psig.

Samples of the effluent containing the test microorganisms were taken from the sampling adapter after 1, 2½, 3, 4, 5, and 6 hours. Duplicate plates of nutrient agar were inoculated with 0.1 ml each, and one ml of effluent was inoculated into 10 ml of nutrient broth. Duplicate sets were prepared, one for incubation at 30 C, the other at 37 C. Results are shown in Table 6.

When the steam mixing jet was not used the temperature of the effluent in the waste tank was not uniform. The drain elbow did not remain hot throughout the test. By comparison, in Test 1 the drain elbow was hot throughout the test, indicating complete circulation of the effluent waste during the sterilization cycle when the steam mixing jet was turned on.

TABLE 6. RECOVERY OF MICROORGANISMS FROM PRETREATMENT TANK STERILIZATION CYCLE, TEST 2[a]

Sample Time, hr	Incubation Temp, 30 C			Incubation Temp, 37 C		
	Nutrient Agar	Nutrient Broth	Subculture of Nutrient Broth	Nutrient Agar	Nutrient Broth	Subculture of Nutrient Broth
Control	TNTC[b]	Positive	TNTC	TNTC	Positive	TNTC
1	3	Positive	TNTC	Neg.	Sl. turbidity	TNTC
2½	5	Sl. turbidity[c]	Neg.	3	Neg.	Neg.
3	5	Positive	TNTC	1	Neg.	Neg.
4	5	Positive	TNTC	1	Sl. turbidity	TNTC
5	6	Positive	TNTC	2	Sl. turbidity	TNTC
6	1	Sl. turbidity	Neg.	1	Neg.	Neg.

a. S. marcescens was not recovered from this test, probably because of adding the culture to 89 C liquid. B. subtilis only recovered.
b. Represents average of duplicate plates.
c. May be due to extraneous material.

3. Test 3

Because the effluent material was not drained from pretreatment tank 2, a sample was taken through the manhole with a sterile bottle before adding the test microorganisms. To insure that the sample was not being contaminated, immediately upon removal of the bottle from the manhole it was immersed in a sodium hypochlorite solution (1000 ppm). Samples were then transferred aseptically to nutrient agar plates, nutrient broth tubes, and lactose broth tubes. The amount of inoculum was the same as in Test 2.

Following the addition of 3½ liters each of B. subtilis and S. marcescens suspensions to the effluent tank, a control sample was taken through the manhole after 10 minutes of mixing with the steam jet. The sampling bottle was immersed in hypochlorite solution upon

removal from the pretreatment tank and transported to the laboratory for assay as outlined above. The tank temperature before addition of the test microorganisms was 59 C.

After heating for 1½ hours the tank contents reached 102 C. At that time the steam jet was turned off and the temperature was held at 102 C for 12 hours. Samples were taken through the sampling adapter after 12 hours and used to inoculate lactose broth tubes, nutrient broth tubes, and nutrient agar plates as described in Test 1.

The elbow containing the sampling adapter on the drain pipe was cold after 12 hours of heating, once again indicating that mixing does not occur within the pretreatment tank unless the mixing jet is turned on.

The pretreatment tank was held at 100 C* for a total of 20 hours. Then a dip sample was taken with a sterile bottle through the manhole and a sample was obtained through the sampling adapter. The data obtained in Test 3 on the pretreatment tank are shown in Table 7.

TABLE 7. RECOVERY OF MICROORGANISMS FROM PRETREATMENT TANK STERILIZATION CYCLE, TEST 3[a]

	Incubation Temp, 30 C (average of duplicate plates)		Incubation Temp, 37 C (average of duplicate plates)		
Sample Time and Location	Nutrient Agar	Nutrient Broth	Nutrient Agar	Nutrient Broth	Lactose Broth
Control tank uninoculated	Positive	Positive	3	Positive	Negative
Control tank inoculated	Positive	Positive	TNTC	Positive	Positive
12 hr at 102 C[b]	Positive	Positive	TNTC	Positive	Positive
20 hr at 100 C	Positive	Positive[c]	1 Pos. 1 Neg.	Positive[c]	Positive

a. B. subtilis growth only.
b. Data represent samples taken through manhole and adapter.
c. One tube of 4 positive.

* Fluctuations in temperature are caused by over- or under-riding of the temperature control mechanism.

4. Test 4

The liquid suspensions of S. marcescens and B. subtilis were added to the pretreatment tank as in previous tests. Following addition of the cultures, the effluent material was agitated for 15 minutes with the steam mixing jet, then samples were taken through the manhole. Duplicate tubes of nutrient broth were inoculated with one ml of sample and lactose broth tubes were inoculated with 5 ml. Duplicate nutrient agar plates were also inoculated with the effluent sample. Two sets were incubated at 37 C and 30 C.

The tank was held at 102 C for 12 hours by using the heating coil only. Following the contact time, samples were taken through the sampling adapter and tested as above (Table 8).

TABLE 8. RECOVERY OF MICROORGANISMS FROM PRETREATMENT TANK STERILIZATION CYCLE, TEST 4

	Incubation Temp, 30 C (average of duplicate plates)		Incubation Temp, 37 C (average of duplicate plates)		
Sample Time and Location	Nutrient Agar	Nutrient Broth	Nutrient Agar	Nutrient Broth	Lactose Broth
Control Tank Inoculated (manhole)	Positive[a]	Positive	Positive[a]	Positive	Positive
12 hr at 102 C (adapter)	Negative	Negative	Negative	Negative	Negative

a. B. subtilis only recovered.

C. RESULTS AND ANALYSIS

The four tests performed on pretreatment tank 2 illustrate some of the problems that can be encountered during the operation of a batch sterilization tank when determining operational procedures and establishing sterilization holding times and temperature requirements. The data indicate: (i) a relatively short holding time (less than 15 minutes) is required for sterilization of vegetative microorganisms at 102 C, (ii) operating the pretreatment tank at higher temperature and pressure should insure sterilization at a shorter holding time (as indicated in Test 1 when the temperature of the effluent reached 116 C), (iii) a holding time between 5 and 12 hours should be sufficient to sterilize spores at 102 C. However, further testing is recommended to confirm the holding time, temperature, and pressure for sterilizing spores.

VI. BIOLOGICAL HAZARDS OF TECHNIQUES AND EQUIPMENT

A. SIMULATED ACCIDENTS

1. Description

A flask containing a liquid suspension of S. marcescens organisms was purposely dropped in Room C, Suite 9, to simulate a laboratory accident. The objective of this simulated accident was to assess and determine (i) if organisms escape from the laboratory room to the hallway, (ii) the interval of time required after an accident for a person to call the building resident engineer or his assigned representative, and for the equipment to be turned off, (iii) the time required for microorganisms to be air-washed out of laboratory room, or for the aerosol to settle, (iv) whether the organisms are carried into the hallway by people evacuating the room after an accident, and (v) whether turning the supply fan off and allowing the exhaust fan to continue running is of any advantage.

Three 1-hour slit samplers were positioned within the laboratory room, and two others were placed, one on each side of the door, in the hallway, to recover microorganisms aerosolized during and following the simulated accident (Figs. 25 and 26).

2. Testing

a. Room Evacuated (Test 1)

A 250-ml flask containing 100 ml of S. marcescens suspension (1.5×10^{10} organisms per ml) was dropped from a height of 56 inches to the cement floor (Kalman finish). No one was permitted in the room during the simulated accident, to prevent gross contamination of the plates in the hallway as the person left the room. The flask, set on top of a deep freeze box, was upset by an attached string that led to the hallway. After the simulated accident the resident engineer was called by telephone. The supply fan to Suite 4 was turned off at the master control panel within 1½ minutes after the drop. The timing was measured under ideal conditions, with the resident engineer sitting at his desk, which is located in a room adjacent to the control panel. Needless to say, prolonged delays in turning the supply fan off would result if the engineer were not available. Once the supply fan was turned off, the negative pressure within the suite increased so that the air-lock doors located on the end of the corridor suddenly opened and tripped the interlocking mechanism. Oversizing of the exhaust fan caused a flow of air greater than desired into Room 4C.

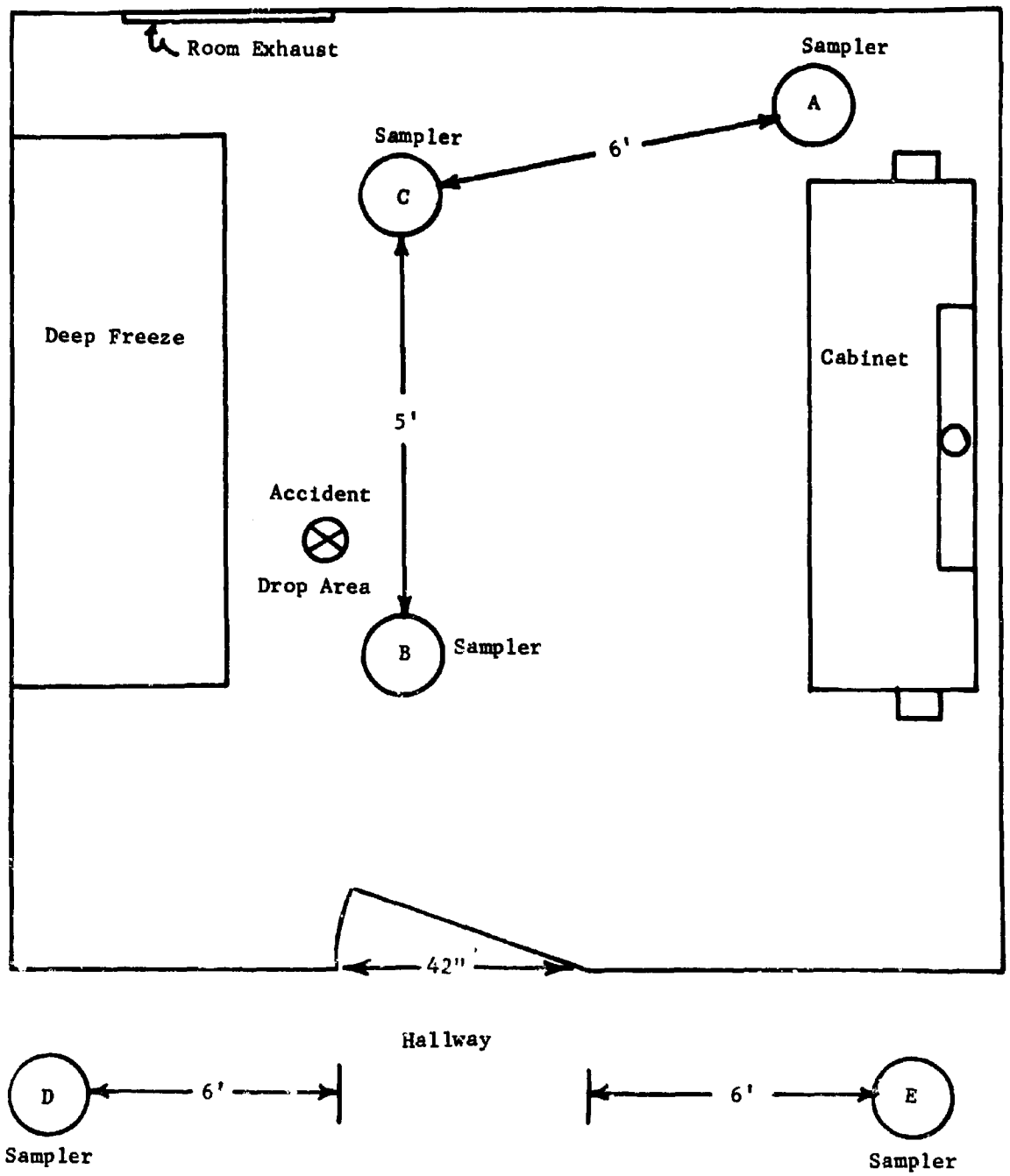

Figure 25. Laboratory Room Layout Sampler Locations.

Figure 26. One Hour Slit Samplers.

Ten minutes after the simulated accident the door was opened to simulate evacuation of personnel. The delay in time was purposeful because an assessment of this hazard was desirable.

Thirty minutes after the accident the supply fan was turned on, bringing the suite's air balance back to its normal operating condition.

The control samples (10 minutes each) taken at all five sampling locations were negative for the recovery of S. marcescens. Recovery of S. marcescens during and following the simulated accident is shown in Table 9, Test 1.

TABLE 9. RECOVERY OF S. MARCESCENS FROM SIMULATED ACCIDENTS

Sampler Location[a]	Average Microorganisms Recovered Per Cubic Foot at Indicated Minutes after Accident			
	1-10 (Control)	10-20	20-40	40-60
Test 1 - Air Supply Fan Off After 1½ Minutes				
A	Neg.	7.3	Neg.	Neg.
B	Neg.	4.4	Neg.	Neg.
C	Neg.	2.8	Neg.	Neg.
D	Neg.	Neg.	Neg.	Neg.
E	Neg. (door opened after 10 min)	Malfunction of equipment		
Test 2 - Air Supply Fan On				
A	0.1	TNTC	0.05	Neg.
B	Neg.	1.2	Neg.	Neg.
C	0.1	1.1	Neg.	0.05
D	0.2	0.1	Neg.	0.05
E	Neg. (2 persons left room in first 15 seconds)	Neg.	0.4	Neg.

a. See Figure 25.

b. Personnel in Laboratory (Test 2)

The previous test was repeated under the same conditions except that the supply fan remained on. Two people were stationed within the laboratory room to duplicate more closely the actual working conditions. One person dropped the flask and left the room immediately, the door was allowed to close, then the other person opened the door and left the room, and the door was again allowed to close. Total time between drop and evacuation of both people was 15 seconds.

3. Results

Data obtained in these tests (Table 9) show that (i) an aerosol is released to the atmosphere during or shortly after the simulated accident,[5] (ii) turning the supply fan off, thereby increasing the negative pressure, confines organisms to the laboratory room; (iii) organisms are carried out of the room to the hallway by personnel evacuating the room; (iv) with the supply fan off and exhaust fan on, the room is free of airborne microorganisms 10 minutes after the simulated accident; and (v) under normal operating conditions (supply and exhaust fans on) the organisms are purged from the room in 30 minutes.

4. Recommendations

On the basis of results obtained in both of these simulated accidents the recommendations are:

1) If such an accident occurs, turn the supply fan to the suite off within the first 5 minutes if possible, and evacuate personnel immediately. If deemed necessary to secure an operation, personnel may enter within 10 minutes if they wear complete protective clothing and respirators. Otherwise, it is advisable to wait the 30 minutes for normal aeration with the supply fan on.

2) In areas where large quantities of infectious materials are to be handled, provide emergency clothing in a readily accessible area to prevent gross contamination of hallway and decontamination change room, and subsequent exposure of other personnel within the suite in adjacent laboratory rooms.

3) Instruct personnel to avoid inhaling after an accident until they have left the room.

4) Personnel assigned to clean up after an accident should wear respiratory protection and should apply decontaminating liquid appropriate for the agent in use[6] by flooding instead of by a forceful spray.[5]

5) Appropriate decontamination procedures should be established.

6) Establish a committee to assess anticipated hazards.

7) If it is determined by subsequent testing that the hazards are severe, an emergency electrical throw switch could be centrally located within each room to turn the supply fan off. If the switch arrangement is not feasible, an audible alarm and light system could be incorporated in each suite and connected to the boiler room, to alert the maintenance man to turn off the supply fan for a particular suite.

5. Precautions

Caution must be exercised in turning off the supply fan with the exhaust operating. Otherwise, a back draft is created through the incinerator stack and fire-box that may result in biological and industrial hazards. If the supply fan is turned off after an accident, close control should be maintained on the interlocking mechanisms of the air lock so that it is set freely to permit air supply to the suite under these conditions.

B. POURING INFECTIOUS MATERIALS DOWN AN OPEN DRAIN

1. Description

The closest drain for introducing a suspension of B. subtilis and S. marcescens into pretreatment tank 2 was a water closet (Fig. 27) in the decontamination change room in Suite 3. The water closet was used in all tests on pretreatment tank 2. The hazard associated with pouring contaminated material into an open drain was evaluated by placing one-hour slit samplers around the immediate area of the water closet.

2. Testing

The cultures were packaged in 500-ml bottles for ease of handling. A 10-minute control sample was taken at each location before the bottles containing the cultures were brought into the area. After the suspensions (500 ml each) of B. subtilis and S. marcescens were poured into the water closet it was flushed one time. This addition and flushing were repeated six times, and after the last addition of the organisms, the water closet was flushed six more times. Time required for addition of the microorganisms and flushings was five minutes. The suspensions were poured from a height of 12 inches above the water level within the water closet, so that some splashing resulted. The height was determined to be that normally used by personnel when pouring into an open drain. During the flushing with water, considerable force was present that produced splashing and turbulence within the closet.

Upon completion of the pouring, two people evacuated the change room in the normal exit manner. Position of slit samplers is shown in Figure 27.

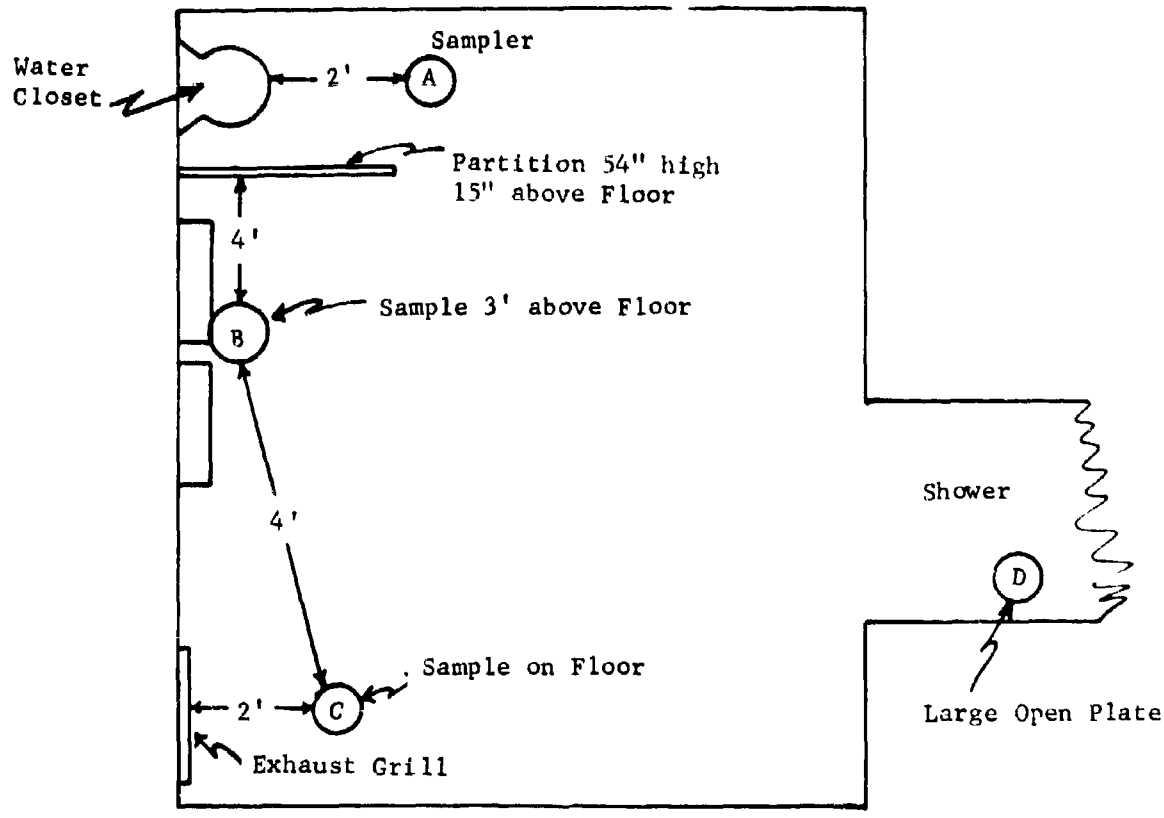

Figure 27. Pretreatment Tank Inoculation Site.

3. Results and Analysis

Recovery of organisms during the pouring operations is shown in Table 10. The recovery of organisms at all sampling locations shows that an infectious aerosol hazard can be caused by dispensing infectious material into an open drain system. Evaluation of the results as presented in the table indicates the hazard to be minimal when expressed as organisms per cubic foot of air. However, the aerosol concentration at the respiratory level of the person pouring was not determined. Consequently, a person could receive an infectious dose of some materials during the initial pouring operation. Some other factors tending to mask the true hazard to this operation are the efficiency of the sampler, air exchange within the room itself, location of exhaust duct, and positioning of samplers.

TABLE 10. RECOVERY OF S. MARCESCENS FROM POURING CULTURE DOWN OPEN DRAIN

Sampler Location (Fig. 27)	Average Number of Microorganisms Recovered Per Cubic Foot of Air at Indicated Minutes			
	Control Before Pouring	During and After Pouring		
	1-10	10-20	20-40	40-60
A - S. marcescens	Neg.	12.5	Neg.	Neg.
B. subtilis	Neg.	13.7	Neg.	Neg.
B - S. marcescens	Neg.	4.1	Neg.	Neg.
B. subtilis	Neg.	4.2	4.2	4.2
C - S. marcescens	Neg.	2.7	Neg.	Neg.
B. subtilis	Neg.	17.7	Neg.	Neg.
D - S. marcescens	Neg.	Neg.	Neg.	Neg.
B. subtilis	Neg.	2.5	Neg.	Neg.

C. BIOLOGICAL CABINETS

1. Description

The potential escape of microorganisms from cabinets with various closure conditions was assessed simultaneously with the testing of the filters. Two slit samplers[3] were positioned on the floor in front of the cabinet, one toward each end.

The cabinets are designed so that they may be used in one of three closure conditions: with glove panel on and gloves installed on the panel; glove panel on, working through open glove ports (no gloves attached); glove panel off (Fig. 12). For all conditions the exhaust blower and manual damper were set to give a reading across the filter as recorded on the Dwyer gauge of 2.8×10^3 linear feet per minute.

In each test the slit samplers were operated in the same position, and the control samples were taken simultaneously with those at the sampling locations along the exhaust duct and at the filter plenum.

2. Physical Arrangement of Cabinets

a. Test 1: Glove Panel On, Gloves On (Suite 4, Room B)

In this test in Suite 4, Room B, one person was in the laboratory and remained there during the entire dissemination and sampling time period.

b. Test 2: Glove Panel On, Gloves Off

The person disseminating the test microorganisms remained in the room (Suite 4, Room B) during dissemination, then evacuated the room 15 minutes after dissemination was completed.

c. Glove Panel Off

(1) Test 3: Suite 1, Room B

The air flow in Room B was reversed by exhausting the air into the hallway. The air flow into the cabinet was 55 linear feet per minute. Generally, the air imbalance within the room, with attendant fluctuation of the air flow into the cabinet, is such that when the door is opened the positive pressure created by the supply fan is great enough to pull airborne organisms out of the cabinet into the room.

Two people walked out of the laboratory room immediately after dissemination; one minute later two reentered and left immediately.

(2) Test 4: Suite 1, Room C

A very strong negative pressure was present in Room C, as shown by the air flow across the open-fronted cabinet. This prevented measurement with the Alnor velometer, and indicated that the room's negative air stream overrode the negative pressure of the cabinet exhaust. The Dwyer gauge reading was 2.3×10^3 feet per minute.

(3) Test 5: Suite 3, Room B

All conditions within the cabinet and room were maintained at operating conditions. The disseminating device in this test was pointed to the front of the cabinet instead of to the rear, which was the position used on all other tests. The results of this test must be interpreted accordingly.

(4) Test 6: Suite 2, Room B

Air pressure measurements within the room indicated the probability of the air flow's being near static. The Alnor velometer reading at the front of the hood was 60 linear feet per minute, and the differential across the filter on the Dwyer gauge was 3.2×10^3 feet per minute.

3. Results

From the six tests performed as outlined above and recorded in Table 11, the following conclusions can be drawn:

1) The increasing order of biological aerosol hazard with the cabinet in varying closure conditions is: (i) glove panel on, gloves installed; (ii) glove panel on, gloves off; and (iii) glove panel off.

2) Any imbalance in the room air flow from the desired condition will produce a hazard in proportion to the air imbalance, regardless of whether the excessive pressure is negative or positive in relationship to that in the hallway (Fig. 28).

3) Opening the room door when the cabinet exhaust air is at minimum flow will produce an aerosol hazard to operating personnel.

4) In Test 1 the sampling results showed that no hazard exists to operating personnel if a cabinet has the glove panel on, gloves installed in the panel, and the cabinet operated under negative pressure.

5) If attached gloves are to be omitted, there is a significant safety advantage in leaving the glove port panel attached, in contrast to operating with it removed.

TABLE 11. RECOVERY OF S. MARCESCENS FROM ROOM AIR WITH VARIOUS CLOSURE CONDITIONS ON BIOLOGICAL SAFETY CABINETS

Test[a]	Cabinet Closure Condition	Slit Sampler Location	Average Number of Microorganisms Recovered Per Cubic Foot of Air Sampled at Indicated Minutes			
			Controls	After Microbial Aerosolization		
			1-10	10-20	20-40	40-60
1	Panel on, gloves on	A	Neg.	Neg.	Neg.	Neg.
		B	Neg.	Neg.	Neg.	Neg.
2	Panel on, gloves off	A	Neg.	0.1	0.05	Neg.
		B	Neg.	0.1	Neg.	Neg.
3	Panel off Room air-flow into hallway	A	Neg.	TNTC[c]	TNTC	TNTC
		B	0.3[b]	TNTC	TNTC	TNTC
4	Panel off Room air pressure strongly negative	A	0.2	TNTC	TNTC	3.0
		B	0.7	TNTC	TNTC	0.6
5	Panel off Normal air Aerosol toward hood front	A	Neg.	TNTC	TNTC	TNTC
		B	Neg.	TNTC	TNTC	TNTC
6	Panel off Static air flow	A	0.6	TNTC	TNTC	0.3
		B	0.1	TNTC	TNTC	2.0

a. See text for test condition details.
b. May be due to background contamination.
c. Too numerous to count.

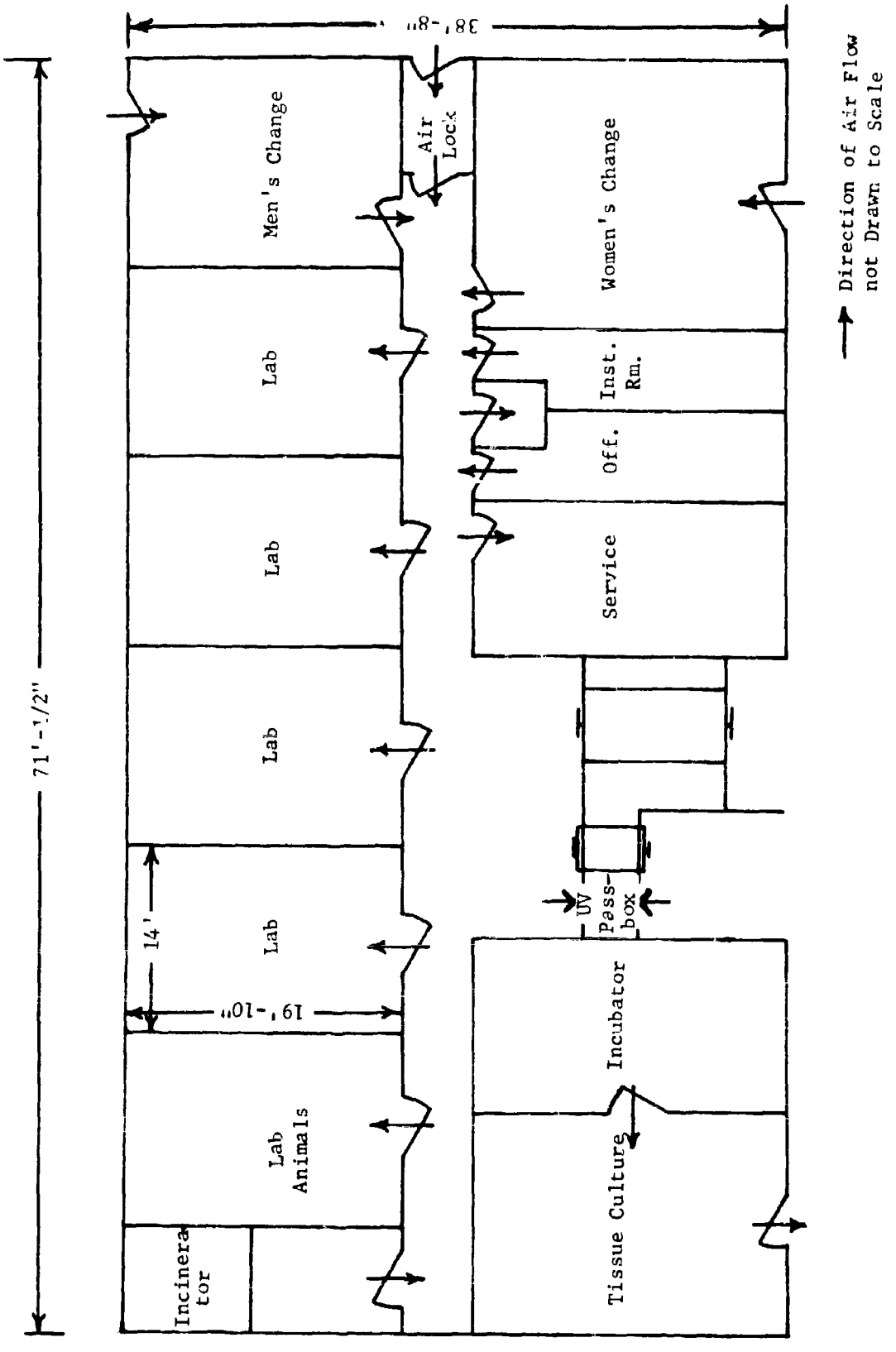

Figure 28. Suite Air Balance (Typical).

F. ELECTRICAL POWER FAILURE

1. Description

An electrical power failure was simulated during the testing of the cabinet filter in Suite 4, Room C. The objective of this simulated electrical failure was: (i) to determine the potential biological hazard to personnel within the laboratory, (ii) to determine if organisms were carried out of the room by personnel evacuating the laboratory room, and (iii) to determine the time required to air-wash the room after complete electrical failure or interrupted service.

Four 1-hour slit samplers were operated around the cabinet and one in the hallway adjacent to the room (Fig. 29).

2. Testing

a. Front Panel On, Gloves Off

The front panel was placed on the biological hood but the gloves were removed during this test. Ten-minute control samples were taken before dissemination of test microorganisms at all sampling locations.

S. marcescens was disseminated within the cabinet in the normal manner for 10 minutes. At the midpoint (5 minutes) of the dissemination, the main power supply to the entire building was turned off, and the electrical service was then supplied by the emergency diesel-powered generator, which started automatically. The diesel generator carried the full electrical load after 22 seconds' delay. Because the exhaust fans to each suite must be restarted manually, from the control panel, a total delay until the biological cabinet returned to normal exhaust rate was 1 minute and 9 seconds, as indicated by both the magnehelic and the Dwyer gauge. The building was maintained on this emergency power for 45 minutes to establish the capability of the generator. From all indications the generator functioned at peak performance. Concurrently with the simulated power failure two people purposely evacuated the laboratory room.

During the period between the time the emergency generator was turned on and the cabinet exhaust returned to normal, the aerosol of S. marcescens was seen flowing out of the open glove ports.

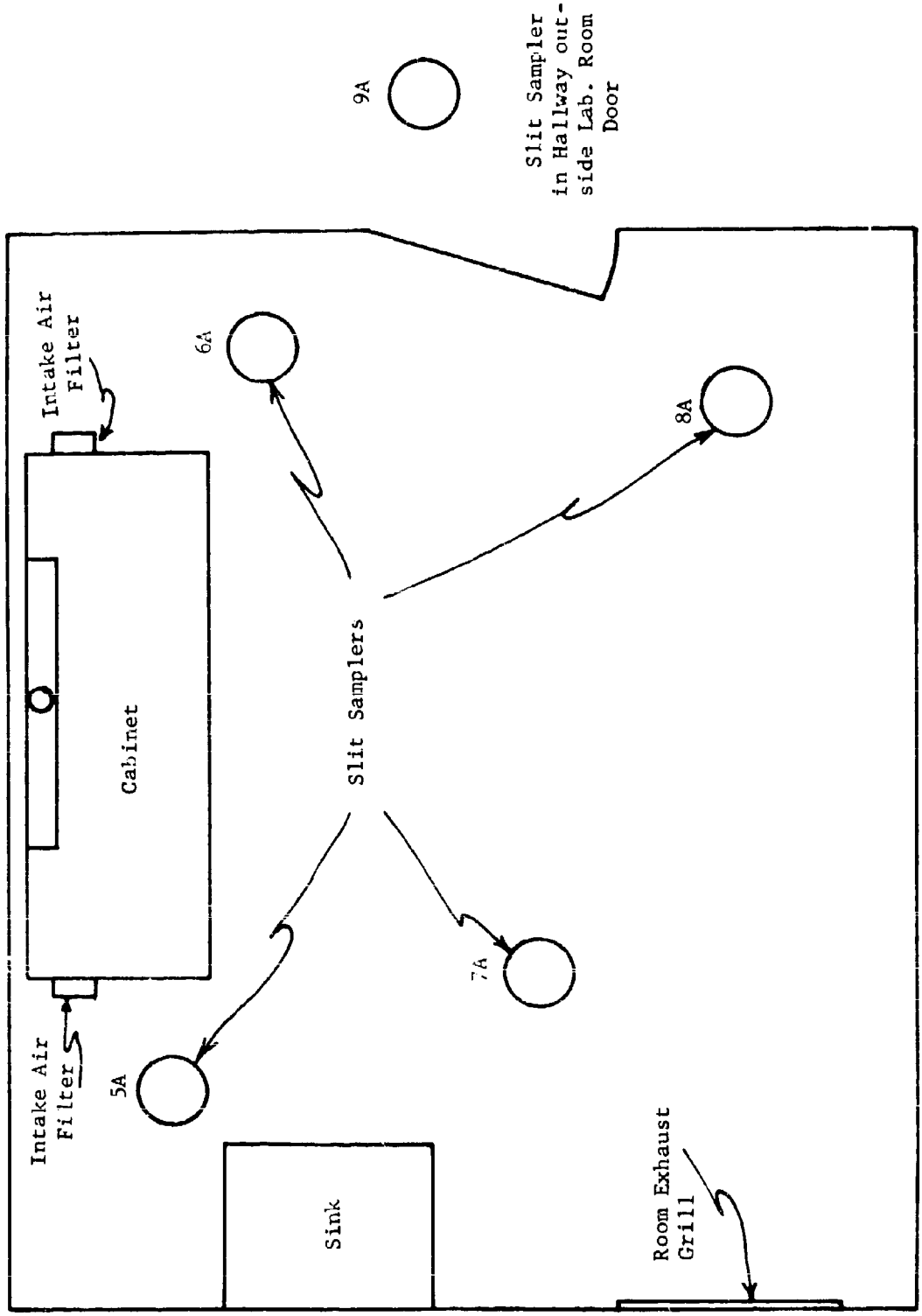

Figure 29. Sampler Locations During Simulated Electrical Failure.

b. Front Panel Off

A second simulated power failure was staged in Suite 2, Room C. Conditions were the same as described above, except that three slit samplers were operated in the laboratory room, the front panel was off, and a very strong positive pressure was present in the laboratory room in relation to the hallway (reversed from the desired).

The power failure was staged at midpoint during dissemination. Nine seconds were required from time of power failure to when the emergency generator carried the full electrical load. The time required to restart the exhaust fan and for the exhaust rate to return to normal was 35 seconds. However, at no time did the cabinet become static, and a slight negative pressure was maintained on the cabinet by fan speed after its interrupted power loss. Following the aerosolization four people evacuated the room.

3. Results and Analysis

The data obtained from these two simulated power failures (Table 12) show that:

1) Microorganisms will escape from a cabinet with the ungloved front panel either on or off; this is not dependent upon whether the cabinet reaches static air pressure conditions. If highly infectious materials were being used, personnel working at an open cabinet would be exposed within a relatively short time (less than 30 seconds) to a significant concentration that, if the infective dose was low, could result in an occupational illness.

2) Organisms are carried to the hallway by personnel evacuating the laboratory room. The number of organisms carried into the hallway depends on the concentration of organisms within the cabinet and the number escaping at time of power failure. Needless to say, the fewer the organisms within the cabinet, the smaller the hazard.

3) The emergency generator functioned very satisfactorily. It maintained adequate power and good air balance throughout all areas.

4) The imbalance in the room air supply and exhaust creates a definite hazard to operating personnel by exhausting microorganisms out of an open cabinet even though negative pressure is maintained.

5) After thirty minutes the aerosol hazard within the laboratory room is minimal. Considering that the test challenge concentration is much greater than that normally used, or expected to be used, in an open-fronted cabinet, the hazard would most certainly be eliminated after 30 minutes' aeration following a power failure.

6) During any loss of power for a short time, the infectious laboratories should be evacuated for a minimum of 30 minutes. However, this is not necessary if the electrical power failure lasts only a few seconds and no loss in negative pressure on the cabinets or room exhaust systems is noted.

TABLE 12. ESCAPE OF S. MARCESCENS FROM VENTILATED CABINET DURING SIMULATED POWER FAILURE

Sampler Location (Fig. 29)	Average Microorganisms Recovered Per Cubic Foot of Air Sampled at Indicated Minutes			
	Control	After Aerosolization		
	1-10	10-20	20-40	40-60
	Glove Panel On, No Gloves			
5 - Room	Neg.	0.6	8.4	Neg.
6 - Room	Neg.	TNTC	0.1	Neg.
7 - Room	Neg.	1.8	Neg.	0.15
8 - Room	0.4[a]	TNTC	TNTC	0.05
9 - Hallway	Neg.	2.0	1.0	Neg.
	Glove Panel Off[b]			
5 - Room	0.6	TNTC	TNTC	6.5
6 - Room	3.0	TNTC	TNTC	Neg.
7 - Room	2.2	TNTC	TNTC	Neg.
9 - Hallway	0.1	12.0	Neg.	Neg.

a. Plate probably contaminated by person who diluted culture and prepared the sample before dissemination.
b. Recovery on the controls probably due to organisms not impinging on agar as predicted in Section IV, A, 3.

E. REFUSE INCINERATOR

1. Description

Each suite is equipped with a refuse incinerator* for disposal of all combustible wastes and animal remains. It is located at the far end of the normal traffic pattern (Fig. 28). The incinerators of adjoining suites are served by a common stack and afterburner. Each suite has a charging room and foreburner in the firebox with a cut-off switch in the charging room. The incinerators, as designed, have the following features: (i) a capacity of 75 poundsper hour; (ii) each burns 3 gallons per hour of No. 2 fuel oil; (iii) stack height is 53 feet 9 inches above ground level; (iv) the operating temperature in the ignition chamber is 870 C, that of the combustion chamber is 760 C, the stack temperature without gas washer is 649 to 760 C; (v) the burners are operated continuously, and thereby eliminate charging of incinerators before the operating temperatures are reached; (vi) the natural draft created by the stack causes a negative pressure within the firebox, which is designed to retain all infectious microorganisms during the loading or burning of contaminated waste materials, (vii) the noncombustible residue is 5 to 10% of the load; (viii) the charging room is under slight positive pressure, to force air into the incinerator whenever the charging door is opened; (ix) the fuel injection system is a positive-feed mechanism.

2. Testing

To assess any biological hazards associated with charging and burning contaminated waste materials, three 1-hour slit samplers were operated in the charging room of the Suite 4 incinerator (Fig. 30). One sampler was placed on a ladder (Sampler A) and one on a box (Sampler B) to sample the air at the breathing level of a person standing or bending to pick up material to be placed in the firebox. The third sampler was placed on the floor (Sampler C), and a fourth was located in the hallway (Sampler D) near the charging room door. All of the plastic plates containing S. marcescens and B. subtilis that had been used in the first 3 days of biological testing were used as the waste material in this test. It was weighed and placed in three no. 10 kraft bags. The bags weighed 2737, 2705, and 1967 grams.

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* Incinerator Engineering Co., 508-510 N. 53rd St., Philadelphia 31, Pa.

Figure 30. Refuse Incinerator.

A 10-minute control sample was taken with each sampler before testing began. The paper bags were carried into the charging room, the door was closed, and the bags were placed on the floor. The foreburner was turned off (this is standard operating procedure) to prevent flash-back from the burner and excessive heat gain within the charging room). The guillotine door to the firebox was raised, at which time a slight negative air pressure was noted on the firebox. Next, the three bags were picked up one at a time from the floor and thrown gently into the firebox. The charging door was closed, the foreburner was turned on, and the worker went from the room to the hallway. During loading, the charging room door was closed and temperature within the room was 54 C in this test. Data on the sampling plates is shown in Table 13. Recovery of microorganisms during the latter part of the control sample in this test indicates that the contamination was probably caused by carrying the bags into the room and placing them on the floor during control sampling.

TABLE 13. RECOVERY OF MICROORGANISMS DURING INCINERATION OF CONTAMINATED LABORATORY WASTE MATERIALS

Sampler	Control 1-10	Average Microorganisms Recovered Per Cubic Foot of Air at Indicated Minutes After Placing Waste in Incinerator 10-20	20-40	40-60
A - B. subtilis	0.7	Neg.	Neg.	0.5
S. marcescens	0.1	Neg.	Neg.	Neg.
B - B. subtilis	0.6	Neg.	0.5	Neg.
S. marcescens	Neg.	Neg.	Neg.	Neg.
C - B. subtilis	0.2	Neg.	0.6	Neg.
S. marcescens	Neg.	Neg.	Neg.	Neg.
D - B. subtilis	0.3	2.0	2.0	2.0
S. marcescens	Neg.	Neg.	Neg.	Neg.

A second incinerator test was performed as above except that plastic poultry bags were substituted for the paper bags. Two plastic bags holding plastic dishes containing S. marcescens and B. subtilis were placed in the incinerator. The bags weighed 1145 and 1043 grams. Two additional plastic bags containing 700 grams each of animal bedding, 10 grams of pelleted S. marcescens (66×10^{10} organisms per gram), and "shoestring" B. subtilis (4×10^{11} organisms per gram) were also placed in the incinerator.

Sampling was performed as noted for the previous tests. There was no recovery of B. subtilis or S. marcescens at any of the four sampling locations.

3. Results

The data from these two tests indicate that a minimal biological hazard exists for operating personnel during loading or incineration of contaminated refuse materials. The paper bags appear to permit escape of spore-forming organisms only during incineration; however, further testing should be done to examine the exterior contamination on the bags. There appears to be no hazard associated with incineration of materials contaminated with vegetative microorganisms. When discarding refuse, the exterior of the containers should be free of contamination.

VII. STERILIZATION WITH BETA-PROPIOLACTONE

A. DESCRIPTION

Suite 3 (area is approximately 32,000 cubic feet) was sterilized with beta-propiolactone (BPL)[7] in the following manner: All equipment, refrigerators, incubators, deep freezes, ovens, etc. were turned off and allowed to reach room temperature. The relative humidity was raised to approximately 80 per cent by disseminating water with Challenger generators (Appendix) strategically located throughout the suite. Fifty sites were marked and spores of B. subtilis (1×10^8 organisms per ml) were sprayed onto the areas with a Sprayon "Jet Pack" spray device.* Control samples of the spore culture before and after spraying, of the cotton swabs, and of the medium were taken before commencing the decontamination. All controls were satisfactory. Manila paper was used to cover the walls adjacent to the Challenger sprayers to avoid peeling the paint. Sensitive equipment was covered in the same manner. The incinerator afterburner in Suite 3 was turned off. Two gallons of BPL were used to decontaminate the suite. Three generators, each containing one gallon of 67% aqueous solution of BPL, were used. They were located at each end and in the center of the suite. Before the relative humidity was increased, all supply and exhaust ventilation was turned off, all doors of individual rooms were opened to the hallway, and all equipment doors and cabinet drawers were left partially opened.

The electricity for the generators was controlled externally. Once a heavy cloud of BPL was obtained throughout the hallway, the exhaust blower to Suite 3 was turned on for one minute to help distribute the BPL vapors throughout the individual laboratory rooms. This technique proved satisfactory in pulling BPL vapors from the hallway to the rooms.

B. SAMPLING

Upon completion of a 2-hour contact time the supply and exhaust fans were turned on. After an overnight purge the air was sampled with a BPL detection tube[8] to determine if BPL vapors were present. All air samples for BPL were negative. After the area was safe to enter, cotton swabs were taken of the 50 previously contaminated areas throughout the suite. The cotton swabs were then streaked on culture medium and incubated at 37°C for 24 hours. The results indicated satisfactory decontamination of Suite 3.

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* Sprayon Products, Inc., 2075 East 65th St., Cleveland 3, Ohio.

C. DISCUSSION

After 15 minutes' dissemination it was necessary to enter the suite because one generator malfunctioned and another was misaligned. To enter the areas containing BPL vapors, a plastic suit, plastic gloves, rubber boots, and a head hood were used. A Scott Air Pack was used as an air supply. Immediately after leaving the suite, the individual showered and washed all protective clothing with water. The Scott Air Pack was wiped down with a water-moistened cloth and hung outdoors to air.

Paint on a door frame peeled slightly where the generator was initially misaligned, and the paint was peeled off the plastic intercommunication box. No other damage due to BPL was observed.

D. RECOMMENDATIONS

The above procedure proved satisfactory for decontamination of Suite 3 with BPL. Therefore, it is recommended that these procedures be followed for decontamination of all other suites. Precautionary measures to follow are:

1) Insure that all electrical circuits to be used have external power control.

2) Generators must be aligned properly to preclude the necessity of a person's entering the suite after dissemination is initiated.

3) During activation of the exhaust blower, insure that the air-lock door is slightly ajar to provide a source of makeup air. Otherwise, the only source of unrestricted air would be through the exhaust stack of the incinerator, and if the incinerator burners were on in the adjacent suite, flash-back and flame damage could result to the incinerator room because the incinerator loading door is not air-tight.

4) The plastic communication box should be covered to prevent paint peeling.

5) If entrance to the suite is necessary before aeration, complete protective clothing and an air supply are highly recommended. Immediately after leaving the suite personnel should shower and wash all equipment to preclude accidental exposure of other personnel to BPL vapors or residue.

6) If BPL is accidentally spilled, it should immediately be neutralized with water.

VIII. CONCLUSIONS

The general design, traffic and air flow patterns, location of laboratory service areas, general laboratory equipment, filtration of all exhaust air, site and operation of the refuse incinerator, batch sterilization of all effluent waste, location of air locks, and location of clean laboratories and corridors with relationship to hot laboratories, are adequate for handling infectious materials.

Tests of cabinet and exhaust plenums with microorganisms showed the filters to have been properly installed within the pockets, filter material to be of appropriate type for the intended job, and that the construction of the filter chambers does not contribute to microorganism escape.

Exhaust blowers carry microorganisms from the cabinets, or from the laboratory room to the exhaust plenums. However, the capacity is greater than necessary to maintain adequate negative pressure. Excess capacity does not contribute proportionally to safety protection.

The exhaust duct network does not permit microorganisms to escape and, from all indications, rupture should not occur unless an excessive force is exerted on the ducts. However, in the event of a duct rupture, as demonstrated in the testing, microorganisms will escape to the atmosphere in spite of the negative pressure maintained in the exhaust system.

Cross contamination does not occur between adjacent legs of the animal cage ventilated exhaust systems. However, the booster blower in the animal cage exhaust system presents certain hazards as currently installed, and modifications should be considered.

The twin plenums for each suite are functioning properly, but under present conditions decontamination of one plenum while the other is operating would be quite difficult unless the recommended modification is made as noted in Section IX.

Additional microbiological testing should be performed on the pretreatment effluent waste tanks to establish sterilization cycles with regard to time, temperature, pressure, amount of effluent waste, and operational features.

The hazards of certain microbiological techniques and equipment were demonstrated to show that human exposure may occur when: (i) personnel have laboratory accidents, (ii) either the air supply or exhaust is imbalanced, (iii) personnel pour infectious material down an open drain, (iv) a cabinet's closure is inconsistent with the risk involved, and (v) electrical service is lost.

The refuse incinerator does not appear to constitute a hazard to personnel loading the incinerator.

The operational design of the air supply and exhaust equipment permits sterilization of the suites independently with beta-propiolactone.

IX. GENERAL RECOMMENDATIONS

The following recommendations are made to maximize safety operations with infectious material:

A. BIOLOGICAL SAFETY CABINETS

The five-inch glove ports should be replaced with eight-inch ports. This would improve reach within the cabinet. The use of attached, arm-length gloves should be considered for work of unusual hazard.

One end plate should be modified to accommodate a pair of gloves and a viewing window to permit two people to work together when inoculating animals or engaging in other operations requiring teamwork.

A pass-through box should be installed on the cabinets to introduce or remove materials whenever the hood is to be used in a closed position. The design of the pass-through box is dependent upon whether the cabinet is to be operated as a Class I or Class III cabinet.

An alternate method for introducing or removing materials is to install a dunk tank on the cabinet.

B. ANIMAL CAGE VENTILATION SYSTEM

To further insure that a hazard does not exist on the discharge side of the exhaust blower, install an in-line Dollinger* or other suitable type of filter between the exhaust ventilation trunk legs and the exhaust blower.

The exhaust blower should be controlled in the same manner as the main plenum blower. That is, if the main plenum blower is off, the exhaust blower is off. This would prevent any possibility of creating a positive pressure on the plenum. However, the plenum blower should be capable of operating without the exhaust blower from the animal cages.

C. EXHAUST PLENUMS

A biologically filtered and valved inlet with its own source of restricted makeup air from the attic should be installed on the dual exhaust plenums to permit decontamination of the filters without interrupting or terminating laboratory research operations.

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* Dollinger Corporation, 11 Centre Park, Rochester, New York.

D. PRETREATMENT TANKS

An additional 8-inch gate valve should be installed on the discharge line on each of the pretreatment tanks. The valve should be located to permit the addition of a steam trap between the existing valve and the one to be added.

Microbiological testing should be repeated to evaluate the holding time, temperature, amount of effluent, and optimum operational cycle for the pretreatment tanks.

An in-line water separator should be installed before the Dollinger filter in the atmospheric vent.

Heating coils and steam jets should be located as close to the bottom of the pretreatment tank as possible.

Multi-opening sampling adapters for testing effluent waste should be installed to check sterility at various locations. Adapter locations should be determined by the tank's design configuration.

Provision should be made for cooling effluent before or during discharge to normal waste system.

Sterilization of pretreatment tank and associated piping should be provided to permit maintenance.

Other recommendations, which would add further protection or refinement to a safety program, can be developed as conditions warrant.

E. ULTRAVIOLET LAMPS

Ultraviolet lamps[9] should be installed in each suite's main air lock to preclude the escape of microorganisms. Additional ultraviolet door barrier lamps may be desirable.

F. MAINTENANCE

Following any major maintenance program the exhaust ducts should be hydrostatically tested as initially performed to insure continued tightness.

G. EFFLUENT WASTE

A routine microbiological sampling of the effluent waste from the pretreatment tanks is necessary after a sterilization cycle and before the waste is discharged into the normal sewage disposal system.

F. AIR BALANCE

A routine air balance check should be established throughout the building.

I. SAFETY EDUCATION

Short safety talks on a weekly basis are recommended to familiarize personnel with the many known microbiological hazards created by equipment, techniques, or procedures.

J. ELECTRICAL CONTROLS

Electrical controls for each suite's supply fan should be relocated. Note other recommendations listed in Section VI, A, 4.

K. STANDARD OPERATING PROCEDURES

Standard Operating Procedures should be posted as guidance in case of a biological accident.

L. DIFFERENTIAL AIR PRESSURE

In future building designs, the differential in air pressures should deal in hundredths of an inch instead of tenths, to eliminate some of the problems mentioned in this report.

M. VENTILATION FOR INCINERATOR CHARGING ROOMS

Air supply should be increased and a proportionately sized exhaust installed in the incinerator charging rooms. Heat gain is excessive.

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APPENDIX

DESCRIPTION OF DISSEMINATING DEVICES

A. PNEUMATIC ATOMIZING NOZZLE

The pneumatic atomizing nozzle (Fig. 1) is a ¼-inch J SS air-supplied, liquid-siphon pick-up type procured from Spraying Systems Co., 3201 Randolph St., Bellwood, Illinois. Air was supplied to the pneumatic nozzle (from building service), at 13 psig, through a Universal air regulating valve (Source: Perfecting Service Co., Charlotte, N.C., Part No. C 200 A); the microorganisms were siphoned (maximum siphoning height 8 inches) through the nozzle from a 250-ml flask. A round spray of the aerosolized microorganisms was produced by mixing the liquid and compressed air externally at the nozzle orifice. The nozzle disseminated 9.2 ml per minute of the liquid suspension of microorganisms and produced an aerosol of particles of 3 to 4 microns mass median diameter, spray distance of approximately 4½ feet, and an 8-inch-diameter spray cloud at 1½ feet from the nozzle.

B. JET SPRAYER

The Jet Sprayer, Challenger, Model 5100 CF is a "cold shearing spraying unit"* procured from the Z&W Manufacturing Corp., 30242 Cleveland Blvd., Wickliffe, Ohio (Fig. 2). A ½-horsepower 110- to 115-volt motor with two-stage centrifugal compressor provides compressed air. The air enters the lower impeller section of the double internal vortex venturi swivel nozzle, creating a reduced pressure and thereby lifting the liquid from the reservoir tank and whirling it clockwise through the central chamber. Simultaneously, air enters the upper impeller in a counter-clockwise direction. As the liquid meets the opposing air stream, the liquid is sheared from the sharp edge of the orifice into droplets with an average mass median diameter ranging from less than one to greater than ten microns. Forty-two per cent of the particles disseminated were less than 6 microns in diameter.

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* No heat or liquefied gas is employed.

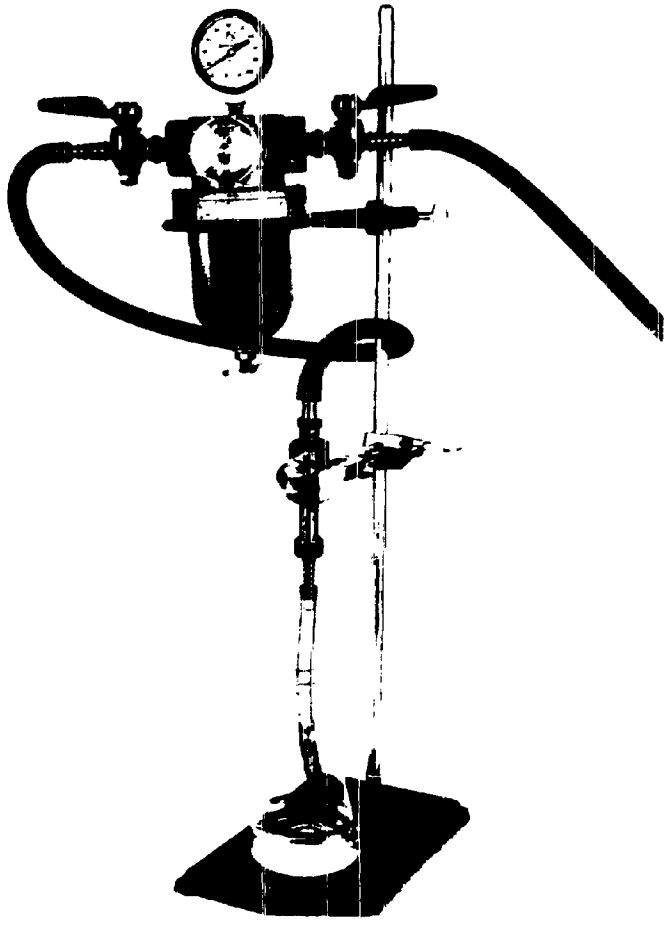

Figure 1. Pneumatic Atomizing Nozzle.

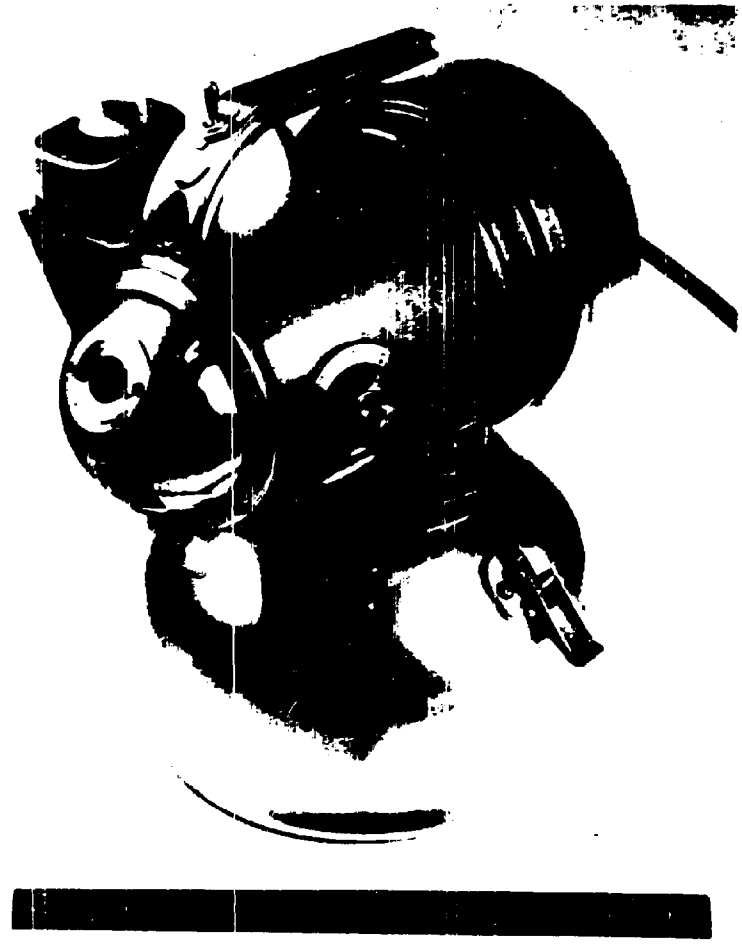

Figure 2. Challenger Sprayer.

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13 ABSTRACT

Operation of buildings and equipment for work with pathogenic microorganisms often involves inherent hazards unknown to personnel responsible for design, construction, budgeting, or research. To a significant extent the facilities and equipment of a laboratory building will aid or deter efforts of operating personnel in maintaining good environmental control and in preventing laboratory infections. The tests reported here were designed to evaluate the microbiological hazards associated with equipment, general building design, construction, operational features, effluent treatment system, and routine research operations in a newly constructed vaccine production facility. The testing procedures and equipment used are described or referenced and the results are tabulated to show the method used to assess the microbiological hazards.

The risks to personnel are characterized, and methods are recommended to improve operation of the building as designed, to modify equipment, and to eliminate hazards.

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